CELLULAR MECHANISMS OF SLEEP HOMEOSTASIS

Studies on Brain Energy Metabolism and Aging

Henna-Kaisa Wigren

Institute of Biomedicine / Department of Physiology
University of Helsinki
Finland

and

The Finnish Graduate School of Neuroscience (FGSN)
Finland

ACADEMIC DISSERTATION

To be publicly discussed
with the permission of the Medical Faculty of the University of Helsinki,
in lecture hall 3, Biomedicum Helsinki, Haartmaninkatu 8,
on February 20th, 2009 at 12 o'clock

Helsinki 2009

Supervised by

Docent Tarja Stenberg
Institute of Biomedicine, Department of Physiology
University of Helsinki, Finland

Reviewed by

Docent Tomi Taira
Neuroscience Center and Department of Biological and Environmental Sciences,
University of Helsinki, Finland

and

Docent Leonard Khiroug
Neuroscience Center,
University of Helsinki, Finland

Discussed with

Professor Irene Tobler
Institute of Pharmacology and Toxicology,
University of Zurich,
Zurich, Switzerland

ISBN 978-952-92-5120-9 (paperback)
ISBN 978-952-10-5292-7 (PDF)
http://ethesis.helsinki.fi
Helsinki 2009-01-28 Yliopistopaino

The important thing in science is not so much to obtain new facts as to discover new ways of thinking about them.

-Sir William Bragg

ABSTRACT

Sleep is governed by a homeostatic process in which the duration and quality of previous wake regulate the subsequent sleep. Active wakefulness is characterized with high frequency cortical oscillations and depends on stimulating influence of the arousal systems, such as the cholinergic basal forebrain (BF), while cessation of the activity in the arousal systems is required for slow wave sleep (SWS) to occur. The site-specific accumulation of adenosine (a by-product of ATP breakdown) in the BF during prolonged waking /sleep deprivation (SD) is known to induce sleep, thus coupling energy demand to sleep promotion. The adenosine release in the BF is accompanied with increases in extracellular lactate and nitric oxide (NO) levels.

This thesis was aimed at further understanding the cellular processes by which the BF is involved in sleep-wake regulation and how these processes are affected by aging. The BF function was studied simultaneously at three levels of organization: 1) locally at a cellular level by measuring energy metabolites 2) globally at a cortical level (the out-put area of the BF) by measuring EEG oscillations and 3) at a behavioral level by studying changes in vigilance states.

Study I showed that wake-promoting BF activation, particularly with glutamate receptor agonist N-methyl-D-aspatate (NMDA), increased extracellular adenosine and lactate levels and led to a homeostatic increase in the subsequent sleep. Blocking NMDA activation during SD reduced the high frequency (HF) EEG theta (7-9 Hz) power and attenuated the subsequent sleep. In aging, activation of the BF during SD or experimentally with NMDA (studies III, IV), did not induce lactate or adenosine release and the increases in the HF EEG theta power during SD and SWS during the subsequent sleep were attenuated as compared to the young. These findings implicate that increased or continuous BF activity is important for active wake maintenance during SD as well as for the generation of homeostatic sleep pressure, and that in aging these mechanisms are impaired.

Study II found that induction of the inducible NO synthase (iNOS) during SD is accompanied with activation of the AMP-activated protein kinase (AMPK) in the BF. Because decreased cellular energy charge (ATP↓:AMP↑) is the most common cause for AMPK activation, this finding implicates that the BF is selectively sensitive to the metabolic demands of SD as increases were not found in the cortex. In aging (study III), iNOS expression and extracellular

levels of NO and adenosine were not significantly increased during SD in the BF. Furthermore, infusion of NO donor into the BF did not lead to sleep promotion as it did in the young. These findings indicated that the NO (and adenosine) mediated sleep induction is impaired in aging and that it could at least partly be due to the reduced sensitivity of the BF to sleep-inducing factors. Taken together, these findings show that reduced sleep promotion by the BF contributes to the attenuated homeostatic sleep response in aging.

ORIGINAL PUBLICATIONS

This thesis is based on the following publications referred to in the text by their Roman numerals:

I. Wigren H-K, Schepens M, Matto V, Stenberg D, Porkka-Heiskanen T (2007). **Glutamatergic stimulation of the basal forebrain elevates extracellular adenosine and increases the subsequent sleep**. Neuroscience. 2007 Jul 13, 147 (3):811-23.

II. Wigren H-K and Porkka-Heiskanen T (2009). **Inducible nitric oxide synthase and AMP-activated protein kinase in basal forebrain during prolonged waking**. Neuroreport. 2009 Jan 7; 20 (1):97-101

III. Rytkönen K-M, Wigren H-K, Kostin A, Porkka-Heiskanen T and A. Kalinchuk (2008) **Nitric oxide mediated recovery sleep is attenuated with aging**. Neurobiology of Aging. 2008 Dec 4 [Epub ahead of print]

IV. Wigren H-K, Rytkönen K-M, Porkka-Heiskanen T (2008). **Aging impairs basal forebrain lactate release and wake promotion during prolonged waking**. Submitted.

ABBREVIATIONS

ACh	acetylcholine
aCSF	artificial cerebrospinal fluid
ADP	adenosine diphosphate
AICAR	5-Aminoimidazole-4-carboaximide-1-β-4-ribofuranoside
AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMP	adenosine monophosphate
AMPK	AMP-activated protein kinase
pAMPK	phosphorylated AMPK
ANOVA	analysis of variance
ATP	adenosine triphosphate
BF	basal forebrain
CX	cortex
DHK	dihydrokainate
EEG	electoencephalography
EMG	electromyography
GABA	gamma-aminobutyric acid
GLUT	glutamate
HDB	nucleus of horizontal limb of diagonal band
HF	high frequency
HPLC	high performance liquid chromatography
LAC	lactate
LDH	lactate dehydrogenase
LF	low frequency
MCPO	magnocellular preoptic area
MK-801	(5R, 10S)-(+)-5-methyl-10,11-dihydro-5H-debenzo[a,d]cyclohepten-5,10-imine hydrogen maleate
NAD^+	nicotenamide adenine dinucleotide (oxidized form)
NADH	nicotenamide adenine dinucleotide (reduced form)
NMDA	*N*-methyl-D-aspartate
NREM	non-rapid-eye-movement sleep
NO	nitric oxide
NOS	nitric oxide synthase
iNOS	inducible nitric oxide synthase
nNOS	neuronal nitric oxide synthase
eNOS	endothelial nitric oxide synthase
PBS	phosphate buffered saline
REM	rapid-eye-movement
SD	sleep deprivation
SI	substantia innominata
SWS	slow wave sleep
SWA	slow wave activity
TBS	tris buffered saline
W	wake

1. REVIEW OF THE LITERATURE

1.1. Regulation of vigilance states

1.1.1. Definition of vigilance states

Electroencephalography (EEG) is used to record oscillations of electric potentials across the cortical surface. In sleep research it is utilized for objective identification of vigilance states. Wakefulness and rapid-eye-movement (REM) sleep are both associated with low amplitude, high frequency EEG signals. REM sleep differs from wakefulness by the presence of muscle atonia, which is why electromyographic (EMG) recordings are taken along with the EEG. Slow wave sleep (SWS), also referred to as non-rapid-eye-movement (NREM) sleep, is detected by the occurrence of high amplitude and low frequency EEG oscillations e.g. slow wave activity (SWA). Identification of vigilance states is based on these differences and performed either manually or by semi-automatic- or automatic scoring systems. As a result, hypnograms representing the variation of vigilance states and their durations during the recording period are produced. In addition to providing means for an objective definition of vigilance states, the EEG rhythms give detailed information on state-specific changes in arousal, behavior and sleep homeostasis (Nunez and Srinivasan, 2006;Achermann and Borbely, 2003) (See section 1.2 for details).

1.1.2. Thalamocortical system

The EEG signal results from summated postsynaptic potentials of vertically oriented pyramidal neurons, and only synchronous co-activation of large neuronal aggregates generates a signal that can be measured with an EEG (Olejniczak, 2006). Conventionally, the term EEG synchronization was assigned to SWS, as the three major synchronized rhythms: sleep spindles (7-15 Hz), delta (1-4 Hz) and slow oscillation (0.5-1 Hz) take place during SWS. However, synchronized rhythms, such as theta (~4-8 Hz), beta (20-30 Hz) and gamma (30-60 Hz) occur also during wakefulness and REM sleep (Steriade and McCarley, 2005). Synchronized EEG rhythms are generated by complex interactions between brainstem, posterior hypothalamic and basal forebrain neurons projecting to thalamus, neocortex and hippocampus. The intracellular properties and reciprocal connections of cortical, thalamocortical and reticular thalamic cells are at the core of generation of the EEG rhythms (Fuentealba and Steriade, 2005). All synchronized sleep

oscillations are associated with steady hyperpolarizations leading to rhythmic bursting of thalamocortical and cortical cells, as well as to inhibition of the transmission of afferent sensory signals via the thalamus. At the transition from sleep oscillations to those of waking or REM sleep, cells become depolarized and their activity changes from rhythmic spike bursts into tonic firing of single spikes.

1.1.3. Arousal systems and the sleep switch

Several neurotransmitter systems with diffuse projections to thalamus and cortex, classically identified as the ascending reticular activating system (Moruzzi and Magoun, 1949) are essential for inducing and maintaining the thalamocortical system at depolarized state. These so called arousal systems (Jones, 2003) are: the serotonergic system of the dorsal raphe nuclei (DR), the noradrenergic system of the locus coeruleus (LC), the histaminergic system of the tuberomammillary nucleus (TM), the orexinergic system of the lateral hypothalamus (LH), the dopaminergic system of the ventral periaqueductal grey matter and the cholinergic system of brain stem laterodorsal tegmental nucleus/pedunculopontine tegmental nucleus (LDT/PPT) and of the basal forebrain (BF). See figure 1.1.3A.

Neurons of the arousal system act in a coordinated fashion to abolish the low-frequency rhythms in the thalamocortical system and promote tonic activity and the appearance of high-frequency oscillations. In contrast to the other arousal systems, which are largely inactive during both SWS and REM sleep, the cholinergic system, which is probably the most important component in EEG activation, is essential also in inducing brain activation during REM sleep (Buzsaki and Gage, 1989;Kleiner and Bringmann, 1996;Detari et al., 1999).

The arousal systems are in turn reciprocally connected with inhibitory GABAergic cells of the hypothalamic ventrolateral preoptic area (VLPO) (Szymusiak and McGinty, 2008). These cells are most active during transition from waking to sleep and during sleep (Szymusiak et al., 1998;Sherin et al., 1996;Gaus et al., 2002;Lu et al., 2002;Gallopin et al., 2000), and the balance of mutual inhibition between arousal systems and the sleep-active VLPO underlies the transitions between vigilance states (McGinty et al., 2004). Sleep-wake transitions in this system are modeled by the electrical flip-flop switch concept (Saper et al., 2005). Instead of gradual transition through intermediate states, the flip-flop switch produces rapid and discrete state

transitions. This ability to become rapidly and fully alert has obvious advantages for the survival of animals. Schematic representation of the arousal systems is presented in Fig. 1.1.3A and the flip-flop model in Fig.1.1.3B.

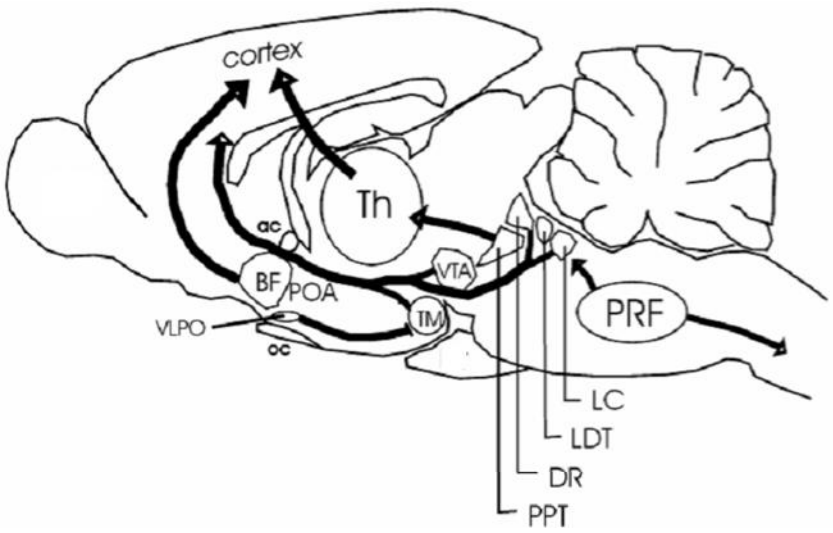


Fig. 1.1.3A. Brain systems regulating sleep and arousal. The figure is adapted from (Stenberg, 2007). Abbreviations: Th=thalamus, ac=anterior comissure, oc=optic chiasma, BF= basal forebrain, VLPO=ventrolateral preoptic area, POA=preoptic area, TM= tuberomamillary nucleus, PPT=pedunculopontine nucleus, DR=dorsal raphe, LDT=laterodorsal tegmental nucleus, LC=locus coeruleus, PRF =pontine reticular formation.

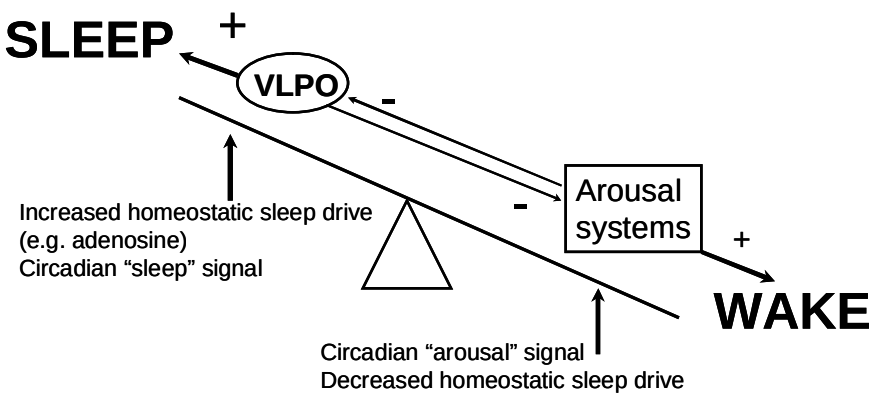


Fig. 1.1.3B. The flip-flop switch.

Unfortunately, a flip-flop switch also has the property of becoming unstable, particularly when the switch is close to its transition point (e.g. during drowsy driving) or when one side of the switch is weakened and becomes less able to inhibit the other side. As an example of such weakening, it has been suggested (Saper et al., 2005) that a cell loss in VLPO during aging may contribute to sleep fragmentation and day-time napping, both of which are frequent phenomena

in the elderly (Ohayon et al., 2004;Cajochen et al., 2006). Accordingly, animals with VLPO lesions wake up more, are unable to go back to sleep while being chronically tired and having brief sleep episodes during wakefulness, all of which are signs of impaired vigilance state consolidation (Lu et al., 2000).

1.1.4. Sleep-wake homeostasis

In addition to the brain machinery that generates oscillations and underlies vigilance state transitions, sleep-wake patterns are regulated by two important factors: circadian and homeostatic. Circadian modulation determines the timing of sleep-wake patterns according to the intrinsic ~24 hour circadian time, which in turn is entrained by external influences, the most important being the light-dark cycle. The circadian arousal signal promotes wakefulness during the active period, while during the inactive period it promotes sleep (Dijk and von Schantz, 2005). The homeostatic process, on the other hand, keeps track on the accumulating sleep propensity during wakefulness, and regulates the amount and intensity of sleep accordingly. If wakefulness is prolonged, sleep pressure increases until sleep is initiated. The higher incidence of SWA and SWS duration in the beginning of recovery sleep, as compared to baseline sleep, are thought to reflect sleep pressure, which dissipates in the course of recovery sleep (Achermann and Borbely, 2003).

According to the two process model of sleep regulation, the interaction between the homeostatic process (S) and circadian process (C) underlies the variations in sleep propensity, the alternation between wakefulness and sleep, SWS intensity, and the timing of REM sleep (Borbely, 1982;Achermann and Borbely, 2003). Schematic presentation of the model is presented in figure 1.1.4.

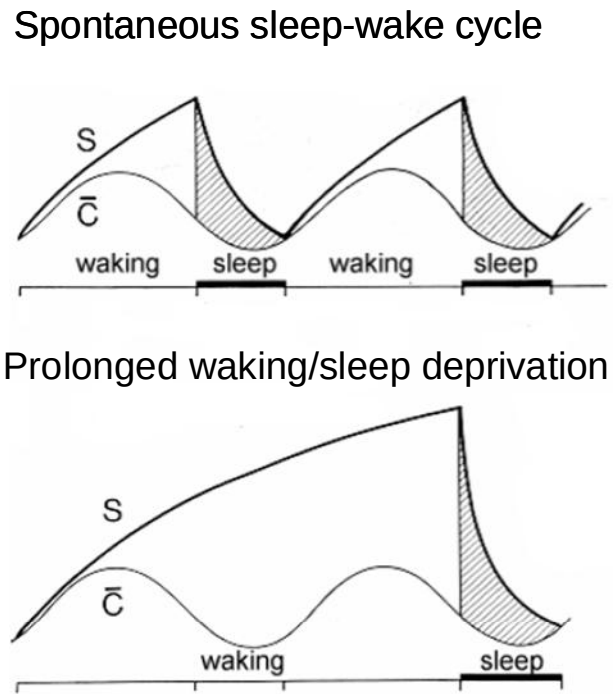


Fig. 1.1.4. Circadian (C) and homeostatic (S) sleep-wake regulation

The model, based on careful measurements of the dynamics of sleep SWA, initially predicted that primarily the duration of prior wakefulness regulates SWA. Recent findings have, however, shown that also the quality of waking is an important factor (Huber et al., 2007;Franken, 2007). Experimental evidence also shows that the regulation of the duration and intensity of sleep can be uncoupled (Tobler and Borbely, 1990;Franken et al., 1991;Cirelli et al., 2005).

Until recently, direct measurements of the build-up of sleep pressure during waking (such as SWA during sleep) could not be performed due to the lack of reliable (EEG) marker. Now it has been shown that waking EEG theta (5-9 Hz) activity, recorded during quiet or immobile waking, could be used as an EEG marker of homeostatic sleep pressure. Theta increases gradually during prolonged waking and was shown to correlate positively with the homeostatic increase of sleep SWA (Finelli et al., 2000;Vyazovskiy and Tobler, 2005).

The molecular mechanisms of circadian rhythmicity in the suprachiasmatic nucleus (SCN), which is the brain's master clock, are fairly well understood (Moore, 2007), while the link between the SCN and the sleep-wake regulating systems is less clear. Direct and indirect anatomical connections between the SCN and the VLPO, and between SCN and the BF, have been found, and suggested to play a role in linking the sleep-wake regulating brain areas to the

circadian system (Saper et al., 2005;Bina et al., 1993). Less is known about the cellular mechanisms underlying the homeostatic sleep regulation. One of the predictions of the two-process model was that accumulation of endogenous sleep-inducing factors during wakefulness and their depletion during sleep would underlie homeostatic sleep regulation (Borbely and Tobler, 1989). At present, several endogenous factors which regulate sleep have been identified (Obal, Jr. and Krueger, 2003;McCarley, 2007;Stenberg, 2007).

A hypothesis by Benington and Heller advanced the idea of sleep factors by stating that the accumulation of sleep pressure during waking is due to the depletion of brain energy stores, and more specifically glycogen (Benington and Heller, 1995). They suggested that adenosine (a by-product of ATP breakdown) is the mediating factor, which accumulates during wakefulness, coupling energy shortage to sleep induction. During SWS brain glycogen levels would be replenished, and thus the function of sleep and its regulation would be tightly coupled to brain energy metabolism. More recently, a hypothesis by Tononi and Cirelli (Tononi and Cirelli, 2006) has suggested that the underlying mechanism of SWS homeostasis is the continuous increase in synaptic strength during wakefulness and synaptic downscaling during sleep SWA. As synaptic activity and particularly plastic changes (including structural reorganization) in the brain are energy consuming (Attwell and Gibb, 2005), it is reasonable to assume that neurons have a larger need for metabolic substances and that they consume more energy during wakefulness than during sleep.

1.1.5. Age-related changes in sleep-wake architecture

Healthy aging is associated with changes in both circadian and homeostatic aspects of sleep. Most of these changes appear already in the middle-ages (Carrier et al., 2001) and likely represent the effects of the intrinsic aging process, rather than being secondary to age-related pathologies. Age-related alterations include: advanced sleep phase, increased sleep latency, decreased total sleep time, reduced SWS intensity, sleep fragmentation, daytime sleepiness, increased napping and reduced homeostatic sleep response to sleep deprivation (reviewed in Miles and Dement, 1980;Bliwise, 1993;Ohayon et al., 2004;Ancoli-Israel and Martin, 2006). These changes present a challenge to our understanding of the neurobiology of vigilance state regulation, but at the same time provide an inviting model to study the basic mechanisms of sleep-wake regulation. It is important to note that the age-related changes may not be just

impairments of the mechanisms, as they are found in the young, but rather reflect the adaptation of the brain to altered biological requirements.

The mechanisms underlying the age-related changes are not well known but they may result from alterations in homeostatic and/or circadian processes. The circadian amplitude of the sleep-wake rhythm is reduced in the aged (Rosenberg et al., 1979;Stone, 1989;van Gool and Mirmiran, 1983) and probably reflects the overall reduction in the circadian function as evidenced by reduced amplitude of core body temperature and melatonin, as well as the circadian modulation of sleep spindles (for reviews see Dijk et al., 2000;Cajochen et al., 2006). It has been proposed that an age-related decrement in the SCN could weaken the circadian arousal signal opposing the homeostatic sleep drive, thus leading to increased sleepiness during waking (Cajochen et al., 2006;Palomba et al., 2008). Some evidence also suggests that increased activation of the cholinergic LDT/PPT (Bassant and Poindessous-Jazat, 2002) during sleep or impaired sleep promotion by the VLPO (Saper et al., 2005) contribute to age-related changes in sleep-wake regulation.

A large body of evidence shows that the homeostatic sleep response to prolonged waking is reduced in aging (Mendelson and Bergmann, 1999a;Mendelson and Bergmann, 1999b;Mendelson and Bergmann, 2000;Shiromani et al., 2000;Gaudreau et al., 2001;Munch et al., 2004;Landolt and Borbely, 2001;Bonnet and Arand, 2007). It is evident from these studies that aged humans and animals alike respond to prolonged waking by increasing sleep SWA, but the amplitude of the increase is reduced as compared to the young. This implies that the core homeostatic mechanisms are intact, but that they operate with reduced capacity. A recent human study concluded that aging is associated with an overall reduction in the maximal capacity to produce sleep (Klerman and Dijk, 2008). Taken together this evidence suggests that both the circadian and the homeostatic mechanisms undergo age-related alterations. However, it remains to be discovered whether the age-related reduction in the homeostatic sleep response reflects a reduced sleep need/ accumulation of sleep pressure or whether the sleep need remains constant, but only the capacity to produce sleep is reduced.

1.2. EEG markers of sleep and arousal

1.2.1. Sleep slow wave activity

EEG activity in the delta (0.5-4 Hz) range, also referred to as slow wave activity (SWA), is routinely used as a physiological marker of SWS intensity and homeostatic sleep pressure (Rosenberg et al., 1976;Borbely et al., 1981;Borbely, 1982). SWA is defined by high amplitude, low frequency (< 4 Hz) activity recorded in the EEG. Recently it was postulated that SWA is important for synaptic homeostasis (Tononi and Cirelli, 2006). According to the hypothesis, the role of SWA is to downscale the net synaptic strength to energetically affordable baseline level. It is assumed that wakefulness is associated with a net increase in cortical synaptic strength, and in order to avoid saturation, a net decrease takes place during SWA. Furthermore, as SWA is a marker of homeostatic sleep pressure, the hypothesis states that the compensatory increase in SWA, which is normally recorded after prolonged waking, should not just depend on the duration of prior waking, but also of the quality of waking, i.e. plastic changes occuring during waking activities. Several experimental studies in recent years have accumulated evidence to support this hypothesis (Faraguna et al., 2008;Vyazovskiy et al., 2008;Huber et al., 2007;Massimini et al., 2007;Vyazovskiy et al., 2007).

It is now acknowledged that SWA is a polymorphic EEG pattern, which reflects more than one cellular phenomenon with distinct mechanisms and sites of origin (Amzica and Steriade, 1998). At least three cellular oscillations are thought to underlie EEG delta activity: cortically generated slow cortical oscillation (< 1Hz), a clock-like thalamic oscillation (1-4 Hz) and a cortical delta oscillation (1-4 Hz). The slow oscillation appears in hyperpolarized cortical cells during SWS and consists of prolonged depolarized (“up”) states associated with neuronal firing and hyperpolarized (“down”) states during which neurons are silent. It occurs synchronously in large populations of neurons and plays an essential role in grouping the other, faster brain oscillations into complex wave-sequences (Steriade, 2006).

Although sensory signals are blocked at the thalamic level during SWS, the intense spontaneous firing of cortical cells during the up-states indicates that the intracortical dialogue and the responsiveness of cortical cells is maintained or even increased during SWS (Dang-Vu et al., 2008). Neuronal oscillations, in general, may lead to transient as well as more permanent changes

in the strength of neuronal connections, and several studies now show that SWA is important for memory processing (see for example Marshall et al., 2006;Born et al., 2006). Slow oscillations also spread into other brain areas and may constitute a neuronal correlate for sleep-dependent memory processing (Molle et al., 2006;Ji and Wilson, 2007;Isomura et al., 2006). These data demonstrate that rather than being a state of quiescence with global inactivation, SWS is an active state during which the brain is engaged in internal processing.

1.2.2. Aging and SWA

During aging SWA decreases and this decrease is particularly evident in the frontal areas (Blois et al., 1983;Ehlers and Kupfer, 1989;Mendelson and Bergmann, 1999b;Bliwise, 1993;Dijk et al., 1989;Landolt et al., 1996;Mourtazaev et al., 1995;Landolt and Borbely, 2001). The cellular correlates underlying the age-related changes in SWS delta are not known but the increased sensitivity of the frontal cortical areas (Raz et al., 1997;Burke and Barnes, 2006;Grill and Riddle, 2002;Uylings and de Brabander, 2002) and the basal forebrain (Sarter and Bruno, 2004;Schliebs and Arendt, 2006) to age-related morphological and functional decrements have been hypothesized to contribute to the reduced "capacity" of brain networks to produce synchronized oscillations. However, it is not known if SWA reduction reflects a reduced homeostatic sleep need.

1.2.3. Theta rhythm and cortical arousal

Oscillations at a theta range are observed at many levels of neuronal organization -from single neurons to large amplitude oscillations recorded with scalp EEG electrodes. Since its first description 1939 in the rabbit (Jung and Kornmuller, 1939), theta has attracted a great deal of attention, particularly because it has been described as a neuronal correlate of numerous cognitive functions such as arousal, learning, memory, attention, information processing and plasticity (for review see (Vinogradova, 1995;Vertes et al., 2004;Buzsaki, 2005;Kahana et al., 2001). In spite of seven decades of intense investigation, no general agreement exists on the primary role or function of theta range oscillations. It is not known whether the theta range activities have a unitary function in all brain areas nor what the actual behavioral correlates of theta are (Vinogradova, 1995;Buzsaki, 2006;Mitchell et al., 2008). Nevertheless, it has been suggested that coordinated theta rhythms facilitate or gate transmission of information between different neuronal systems (Young and McNaughton, 2008).

In humans EEG theta activity is usually defined as an activity occurring in the 4-8 Hz range (IFSECN, 1974). It is most extensively studied in the frontal midline derivations, where it increases during performance of different cognitive tasks. Numerous publications link the frontal midline (FM) theta to increasing mental effort, particularly during working memory tasks (Young and McNaughton, 2008) suggesting that FM theta may reflect increased activity of the attentional systems (Sauseng et al., 2007). Another line of research has shown that EEG theta activity (when recorded during resting wakefulness/immobility) increases together with sleepiness and decreased alertness particularly in the frontal areas (Lorenzo et al., 1995;Cajochen et al., 1995;Cajochen et al., 2002;Aeschbach et al., 1997;Aeschbach et al., 1999 ;Finelli et al., 2000;Strijkstra et al., 2003). Based on these observations, waking theta was assigned "the EEG correlate of sleep pressure during waking" comparable to SWS delta, which is the correlate of sleep pressure in the sleep EEG. It is not known whether these two types of theta (the FM theta and the sleepiness related theta) are distinct phenomena or whether they both simply reflect the increased compensatory effort of the attentional system to maintain arousal during challenging situations.

In animals, theta band designation ranges from ~4-12 Hz depending on species, method of recording and location of electrodes (Winson, 1972;van Lier et al., 2003). In rodents, theta is clearly visible during active brain states such as exploratory behavior, immobile attentive waking and REM sleep (Vanderwolf, 1969;Coenen, 1975;Bland, 1986;Vanderwolf, 1988). Increases particularly in the higher theta frequencies (> 8 Hz) are recorded during active exploration (locomotion and rearing) as compared to grooming or immobility when low frequency theta dominates (Kramis et al., 1975;Kramis et al., 1975;Huber et al., 2007;Vyazovskiy and Tobler, 2005;Young and McNaughton, 2008). In line with human studies (Finelli et al., 2000), a recent rat study showed that an increase in the low frequency EEG theta during quiet wakefulness correlates positively with increased homeostatic sleep drive in the course of sleep deprivation (Vyazovskiy and Tobler, 2005).

Rodent theta is most extensively studied in the hippocampus (Bland, 1986;Buzsaki, 2005) but can also be recorded in many cortical and subcortical areas (for review see (Kahana et al., 2001). There has been controversy over whether the extra-hippocampal theta is a consequence of the activity of the local oscillators or whether it is due to passive volume conduction from the

hippocampus (Feenstra and Holsheimer, 1979;Colom et al., 1988;Talk et al., 2004). Several studies in both humans and animals now support the existence of independent theta generators (for review see Kahana et al., 2001).

1.2.4. Cholinergic modulation of theta

Neocortical and hippocampal theta rhythms are strongly modulated by acetylcholine (Vanderwolf, 1975;Vanderwolf, 1988). It is well established that the cholinergic input from the medial septum/diagonal band of Broca (MS-DBB) is important in the generation of the hippocampal theta (Winson, 1978), while the cholinergic input of the basal forebrain (BF) has been suggested to modulate the cortical theta activity (Lee et al., 2005;Jones, 2008). Results from lesions of the MS-DBB, which disrupt theta rhythms in the hippocampus, but not in the cingulate cortex, support the notion that separate pathway exists for cortical theta (Borst et al., 1987;Borst et al., 1987). MS-DBB and the BF both receive strong inputs from the ascending reticular activating system, and stimulation of these ascending pathways evokes hippocampal and cortical theta (Detari et al., 1999; Detari et al., 1997;Semba et al., 1989;McNaughton and Sedgwick, 1978;Vertes, 1982;Vinogradova et al., 1995;Vertes and Kocsis, 1997).

1.2.5. Theta in aging

Due to the diverse theta definitions used in the literature, reports on age-related changes in EEG theta activities are not concise (for review see (Rossini et al., 2007). Human resting EEG full band theta power is generally reported to increase with age whereas the task-dependent increases in the FM midline theta are reduced or absent in aged subjects (McEvoy et al., 2001;Cummins and Finnigan, 2007) (Babiloni et al., 2004). In senescence-accelerated mice the peak frequency of EEG theta during brain waking and REM sleep was lower than in the young (Colas et al., 2005). This was hypothesized to reflect the reduced cognitive performance, also reported in these mice. In old rats the peak frequency of acetylcholine-evoked hippocampal theta rhythm was decreased (Abe and Toyosawa, 1999). This could be a consequence of the age-related changes in the firing rate of septal and brainstem cholinergic neurons (Apartis et al., 2000;Bassant and Poindessous-Jazat, 2002). Treatment of rats with scopolamine (cholinergic agonist) increased high frequency EEG activation (8-20 Hz, including theta) in young rats while the response was attenuated in old rats (Buzsaki et al., 1988a).

1.3. Brain energy metabolism

1.3.1. Activation coupling

Most of the energy consumed by the brain is utilized to sustain neuronal activity (Attwell and Laughlin, 2001). Arising from the early studies of Sherrington (Roy and Sherrington, 1890), the basic principle of brain energy metabolism states that the brain metabolic responses are spatially and temporally coupled to neuronal activity. Subsequent experimental studies have shown that local energy consumption increases with increased neuronal activity and decreases with decreased activity (Sokoloff, 1977;Clarke D.D. and Sokoloff L., 1994), the strongest association being to excitatory glutamatergic neurotransmission (Attwell and Gibb, 2005). This is why the fast availability of glucose and adenosine triphosphate (ATP) are essential for proper brain function.

Glucose is transported directly from the blood or derived from glycogen stores located mostly in the astrocytes. Free energy, liberated during glucose combustion (glycolysis and tricarboxycylic acid (TCA) cycle), is trapped in high-energy phosphate bonds of ATP (electron transport and oxidative phosphorylation in mitochondria). Hydrolysis of ATP into adenosine monophosphate (AMP) via adenosine dipophosphate (ADP) is the main source of cellular energy. The concentrations of ATP and AMP are carefully regulated to favor ATP hydrolysis, and even a small decrease in ATP will lead to a relatively high increase in AMP. The concentration of AMP is in turn coupled to several cellular pathways evolved to preserve the cellular energy charge (AMP: ATP ratio). When neuronal activity exceeds the ability of the circulating blood to provide enough glucose for activated neurons: 1) supplemental energy substrates are mobilized, 2) energy consuming processes are inhibited and 3) energy restorative processes are activated.

1.3.2. Lactate and brain activation

Under normal conditions energy is derived from oxidation of glucose beginning with glycolysis and ending with oxidative phosphorylation. Traditionally these two processes were viewed to be strictly coupled with glycolysis providing the necessary substrate (pyruvate) for oxidative phosphorylation. In the brain, however, it was noticed that during neuronal activation excess glycolysis takes place even in the presence of adequate oxygen supply (Raichle et al., 1970;Fox and Raichle, 1986;Fox et al., 1988;Raichle and Mintun, 2006) and that these glycolytic bursts

lead to production of lactate in astrocytes (Pellerin and Magistretti, 1994;Sibson et al., 1998). These findings contrasted the old and widely accepted dogma that lactate production is harmful and only occurs during anaerobic glycolysis. A reassessment of the old literature and new experimental evidence accumulated over the past decades indicated that lactate is used as an oxidative energy substrate in the brain (for review see Gladden, 2004 and Schurr, 2006).

A model known as the astrocyte-neuron lactate shuttle hypothesis (ANLSH) formulated by Magistretti and Pellerin in 1994 (Pellerin and Magistretti, 1994) and refined in (Pellerin et al., 2007) proposed that during increased glutamatergic activity, it is the glycolytic production of lactate in astrocytes, which provides the additional energy needed to fuel increased neuronal activation (for detailed description see figure 1.3.2). Since the introduction of the ANLSH there has been a virtual explosion of publications on the topic, many supporting its basic assumptions. However, as it challenged the old dogma of glucose being the sole energy substrate for the brain, it was not accepted without controversy (against: Chih et al., 2001;Hertz, 2004; Chih and Roberts Jr, 2003;Korf, 2006 for : Schurr, 2006;Pellerin et al., 2007) and the question of whether lactate or glucose is the sole energy substrate of the brain was fiercely argued (for discussions of the debate see Pellerin et al., 2007 and Gladden, 2004). Notwithstanding the specific details of lactate production and its relative significance, it is clear from experimental studies performed by different methods that brain activation leads to lactate production in both animals and humans (Kuhr and Korf, 1988;Hu and Wilson, 1997;Ros et al., 2006;Caesar et al., 2008;Prichard et al., 1991;Sappey-Marinier et al., 1992;Urrila et al., 2003).

A work by Kasischke and collegues (Kasischke et al., 2004), performed by two-photon fluorescence imaging of NADH upon neuronal activation, demonstrated a clear spatial and temporal segregation of neuronal oxidative energy metabolism and of astrocytic aerobic glycolysis. This finding together with the work of Hu and Wilson, 1997 and Mangia et al., 2003 show that upon activation, extracellular lactate levels initially decrease due to increased neuronal demand of oxidative energy substrates and that this decrease is later followed by a substantial overshoot of extracellular lactate due to astrocytic glycolysis. As most *in vivo* studies have low temporal resolution, they most likely reveal only the late lactate overshoot (Pellerin and Magistretti, 2004). However, the work by Kasischke and colleagues drove Pellerin and Magistretti to revise the original ANLSH (Pellerin et al., 2007), and it is now clear that the

original notion of glutamate uptake being the only signal for coupling neuronal activity to glucose consumption is not the whole truth. It is likely that neuronal ionic movement is the initial signal, which induces the increase in neuronal oxidative metabolism (extracellular lactate decrease) followed by elevation in astrocytic glucose consumption (extracellular lactate increase) (Schurr, 2006) . Furthermore, a recent *in vivo* microdialysis study demonstrated that an activity-dependent lactate increase in the rat cerebellum occurs independently of glutamate uptake but is dependent on glutamate receptor AMPA activation (Caesar et al., 2008), clearly indicating that post-synaptic activation of the lactate response is possible (Kasischke, 2008).

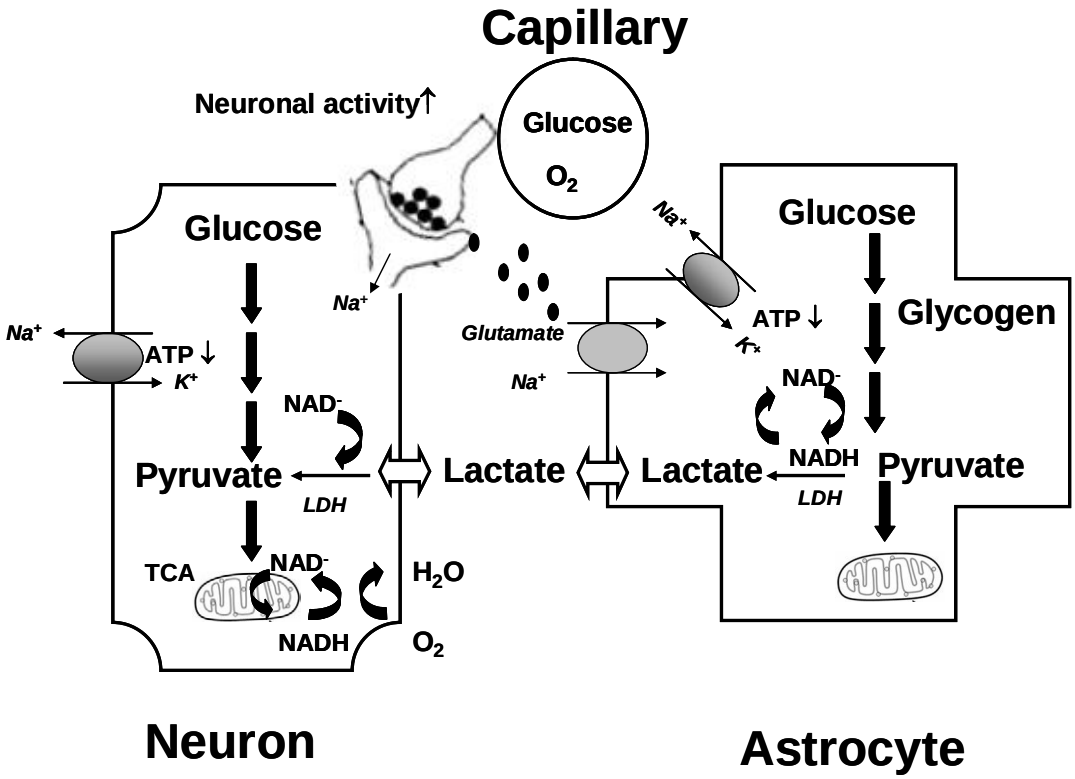


Fig. 1.3.2. Mechanisms of activity dependent lactate production according to ANLSH (adapted from (Pellerin et al., 2007).

1. Neuronal activity first leads to considerable energy expenditure in the postsynaptic neuron due to re-establisment of ionic gradients by Na^+/K^+ ATPase. As a consequence, oxidative phosphorylation is activated and leads to the early decrease in the mitochondrial NADH levels. Subsequent enhancement of the tricarboxycylic acid (TCA) cycle re-produces NADH for oxidative phosphorylation and supports enhanced ATP production. Increased pyruvate ulitization by the TCA leads to decreases in cytoplasmic pyruvate making the conditions favorable for enhanced glucose and lactate use, and causes the early dip

measured in extracellular lactate levels. It is thought that neuronal lactate dehydrogenase (LDH) favors the formation of pyruvate from lactate.

2. Uptake of glutamate into astrocytes via Na^+ -dependent glutamate transporters activates Na^+/K^+ ATPase and conversion of glutamate into glutamine. Both processes are energy demanding and stimulate glucose uptake and lactate production. Lactate is formed from pyruvate in a reaction catalyzed by lactate dehydrogenase (LDH) with concomitant regeneration of nicotenamide adenine dinucleotide (NAD^+) from NADH. Lactate is moved into extracellular space along its concentration gradient by monocarboxylate transporters (MCTs). Increases in extracellular lactate during glutamatergic stimulation constitute the late and tonic phase of the lactate response.

3. Lactate is transported from the extracellular space into neurons where it is transformed back to pyruvate by LDH and used as an oxidative energy fuel in neurons. Lactate production in the late phase replenishes the extracellular pool and helps to sustain neuronal energy needs as activation persists. Upon strong and long lasting stimulation glycogen stores are mobilized to support continuous lactate production.

1.3.3. Lactate from glycogen stores

Upon strong and long-lasting stimulation or during hypoglycemia, when blood-derived glucose availability decreases, astrocytic glycogen stores almost exclusively provide the necessary energy for activated neurons (Brown and Ransom, 2007). In such situations, lactate derived from glycogen is the predominant pathway for energy production. Neurotransmitters other than glutamate and neuromodulators such as adenosine, ATP and nitric oxide also enhance glycogenolysis i.e. lactate production in astrocytes and its utilization by neurons (Sorg and Magistretti, 1991;Sorg et al., 1995;Almeida et al., 2004;Uehara et al., 2008).

1.3.4. Adenosine couples energy shortage to neuronal inhibition

Adenosine is a neuromodulator which couples energy shortage to neuronal inhibition, sleep induction and neuroprotection (Dunwiddie and Masino, 2001;Latini and Pedata, 2001;Basheer et al., 2004;Porkka-Heiskanen et al., 2002). It is formed from ATP, both intra- and extracellularly in a reaction catalyzed by a chain of nucleotidases, the rate-limiting step being the conversion of AMP into adenosine by 5' –nucleotidases. Effects of adenosine are mediated extracellularly via membrane bound receptors. Extracellular adenosine concentration in the brain increases in an activity-dependent manner and in situations when ATP demand exceeds production.

There are four subtypes of G-protein coupled adenosine receptors (A_1, A_{2A}, A_{2B}, A_3), of which the A_1- and A_{2A} have been widely studied and the most likely candidates for mediating sleep induction (Haas and Selbach, 2000;Fredholm et al., 2005a;Stenberg, 2007). Stimulation of pre-synaptic A_1 receptors inhibits the release of all classical neurotransmitters; the most prominent inhibitory actions generally reported at glutamatergic synapses (Dunwiddie and Hoffer, 1980;Kocsis et al., 1984;Rebola et al., 2005). Postsynaptic A_1 receptor stimulation inhibits calcium influxes, NMDA receptors and potassium currents, and thus favors membrane hyperpolarization. Recent data also suggest a role for the extrasynaptic A_1 receptors in regulating energy metabolism and glial function (Haberg et al., 2000;Dare et al., 2007). A_1 -receptor - mediated effects on intracellular calcium signaling and transcription factor activation have also been demonstrated (Basheer et al., 2001;Ramesh et al., 2007;Basheer et al., 2002). Less is known about the functions of A_{2A} –receptors, but in some areas A_{2A} –receptors enhance neurotransmitter release and modulate extracellular adenosine concentration (Pinto-Duarte et al., 2005;Sebastiao and Ribeiro, 1996;Fredholm et al., 2005b). It is generally assumed that at basal extracellular adenosine concentrations both high-affinity adenosine receptors (A_1, A_{2A}) are tonically active.

Extracellular adenosine levels are governed primarily by two mechanisms: 1) conversion of extracellular ATP into adenosine and 2) facilitated diffusion of adenosine between intra- and extracellular space via equilibrative nucleoside transporters (ENTs). The question of whether intra- or extracellular ATP breakdown is the main source of increased extracellular adenosine is still under debate (Cunha, 2008). Formation of adenosine in the extracellular space is based on observation that most cells release ATP and are endowed with ecto-nucletidases (Fields and Burnstock, 2006;Zimmermann, 2000). Adenosine can also be transported into the extracellular space. Under normal conditions intracellular adenosine concentrations are low due to the relatively high adenosine kinase activity; therefore the net flux of adenosine is inwardly directed. An outward flux of adenosine into the extracellular space via ENTs is usually initiated when ATP demand exceeds production. Because intracellular ATP concentrations are extremely high, a 1% conversion of ATP to adenosine would result in a 100-fold intracellular adenosine increase, and if the intracellular adenosine level exceeds the extracellular level then corresponding outward flux via ENTs occurs. Also nitric oxide has been shown to increase extracellular adenosine levels (Rosenberg et al., 2000). The exact mechanisms of NO-mediated increase in extracellular

adenosine are not known but it may be due to inhibition of adenosine kinase and/or oxidative energy production.

Clearance of extracellular adenosine occurs mainly through ENTs when intracellular levels decrease. The main metabolic pathways contributing for clearance of adenosine are the formation of AMP by adenosine kinase and the irreversible breakdown to inosine by adenosine deaminase. Adenosine kinase is enriched in neurons while adenosine deaminase is abundant is astrocytes. The third enzyme, S-adenosyl-homocystene hydrolase (SAHH) converts adenosine to S-adenosyl-homocysteine, but this pathway may not be of significance in the brain. Schematic presentation of the main intra- and extracellular pathways for adenosine formation, transport and receptor action is shown in figure 1.3.4.

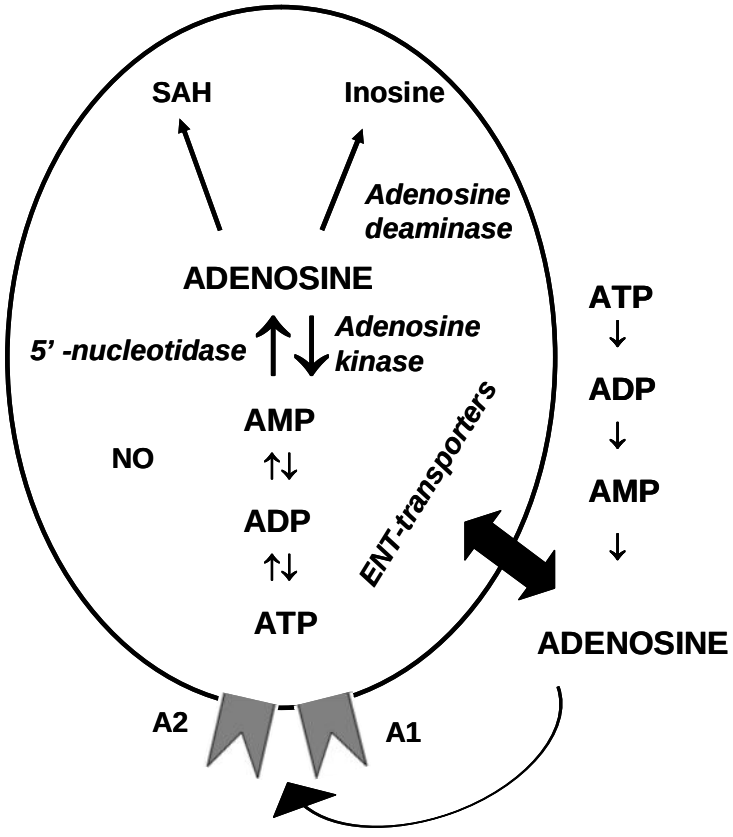


Fig. 1.3.4. Schematic presentation of the main intra-and extracellular pathways for adenosine formation, transport and receptor action.

1.3.5. AMP-activated protein kinase –the cellular energy fuse

The AMP-activated protein kinase (AMPK) is an ubiquitous metabolic enzyme present in almost all cells and organisms (reviewed in Kahn et al., 2005;Hardie et al., 2006;Carling, 2007). When activated by increases in cellular AMP it promotes energy restoration and inhibits energy consumption. AMPK exists as a heterodimer of catalytic α subunits and regulatory β- and γ subunits. It is activated by phosphorylation via upstream kinases LKB1 or calmodulin-dependent kinase kinase (CaMKKβ) at threonine 172 (T172) of its α subunits. The phosphorylated form of AMPK can be detected by specific antibodies and this method has been widely utilized to measure AMPK activation. In the brain AMPK is activated by changes in the cellular energy charge (AMP:ATP ratio), metabolic stress or neuronal-activity mediated rise in intracellular Ca^{2+} (Kuramoto et al., 2007). Schematic presentation of the AMPK activation is presented in figure 1.3.5.

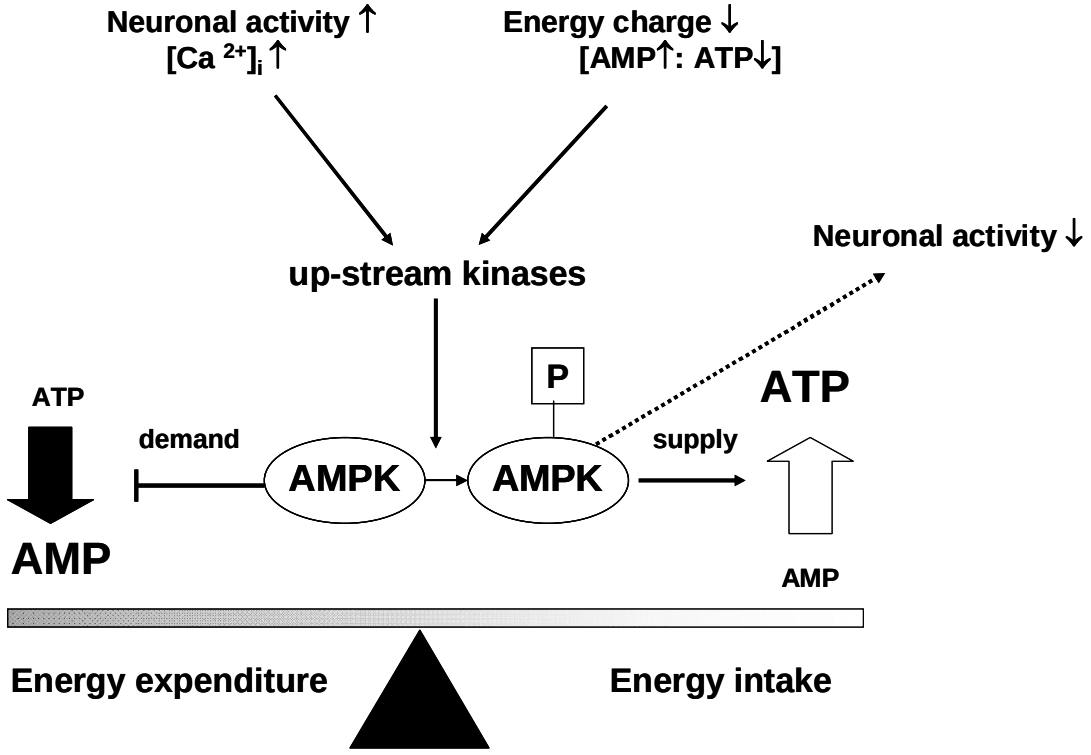


Fig. 1.3.5. AMPK as the cellular energy fuse.

1.3.6. Nitric oxide inhibition of oxidative energy metabolism

Nitric oxide (NO) is a gaseous signaling molecule involved in several cellular functions including neurotransmission, regulation of blood flow and immune response (reviewed in (Guix et al., 2005;Calabrese et al., 2007;Garthwaite, 2008). It is generated from L-arginine in a reaction catalyzed by a family of NO synthases (NOS), namely neuronal - (nNOS), endothelial- (eNOS) and the inducible NOS (iNOS) (Alderton et al., 2001). Of these, nNOS and eNOS are constitutively expressed in the brain, while iNOS is induced during challenges such as immunological response, immobilization stress or sleep deprivation (Murphy, 2000;Madrigal et al., 2001;Kalinchuk et al., 2006a) and can generate considerable amounts of NO. NO has prominent inhibitory effects on mitochondrial energy production. These effects are reversible or irreversible, depending on NO concentration.

NO binds to soluble guanylyl cyclase and to the mitochondrial enzyme cytochrome *c* oxidase. NO interacts with guanylyl cyclase to increase cyclic guanylyl monophosphate (cGMP) concentrations, thus affecting intracellular signaling cascades. The binding of NO to cytochrome *c* oxidase decreases its affinity to oxygen, leading to impaired ATP production. In addition, and particularly in pathological situations, NO inhibits almost all components of the mitochondrial energy production machinery (Cooper, 2003;Palacios-Callender et al., 2007;Moncada and Bolanos, 2006).

However, there are differences in the sensitivity of different cell types to NO-mediated inhibition of cellular respiration. In both astrocytes and neurons, NO incubation profoundly decreases oxygen consumption, but in astrocytes NO also enhances glycolysis (Almeida et al., 2001). The activation of glycolysis by NO is cGMP dependent and requires prior inhibition of mitochondrial respiration. The decreased cellular energy charge activates AMPK and leads to enhancement of glycolysis as demonstrated by activation of 6-phosphofructokinase-1-kinase (PFK1), a key rate limiting step of the glycolytic pathway (Almeida et al., 2004). This response is suggested to have a neuroprotective role (Bolanos et al., 2008).

NO is thermodynamically unstable, which leads to spontaneous reactions with other molecules. For example, oxidation, nitrosylation or nitration of proteins has been reported (Davis et al.,

2001). When NO is produced in excess, such as during pathological insults or inflammation, it forms toxic compounds belonging to a family known as “reactive nitrogen species” (RNS), which cause cellular damage and nitrosative stress. Increased production of NO and inability to reduce the production of RNS is thought to underlie the pathogenesis of neurodegenerative diseases and may also be a factor in healthy brain aging (Bolanos et al., 2004;Guix et al., 2005;Nakamura et al., 2007).

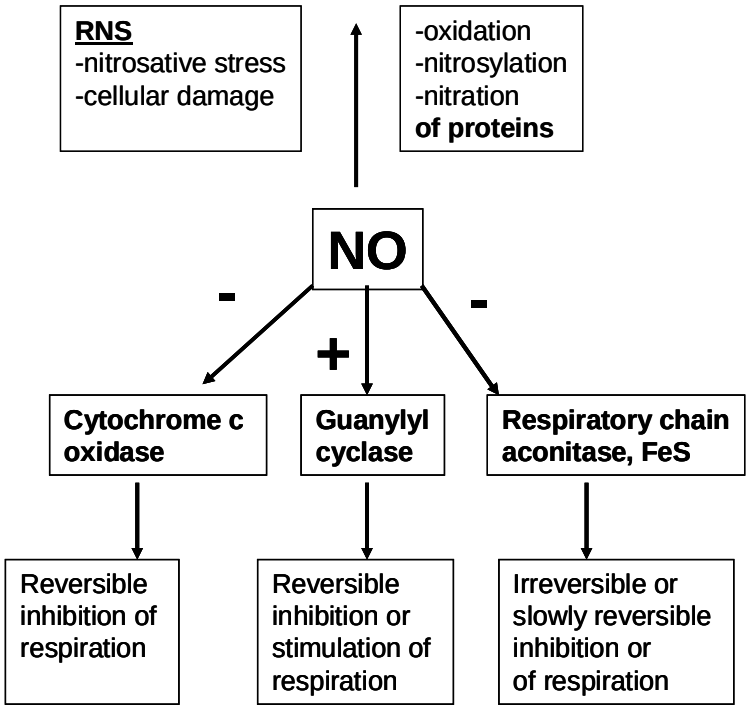


Fig. 1.3.6. NO inhibition of energy metabolism

1.3.7. Metabolic changes in brain aging

Generally with aging, decreases in brain energy metabolism are reported (Hoyer, 1985;Petit-Taboue et al., 1998;Hoyer et al., 2004;Leenders et al., 1990;Toescu and Xiong, 2004). There is, however, no global pattern of decrease (Moeller et al., 1996) and the age-related changes are often subtle and site-specific: the frontal cortical (Raz et al., 1997;Martin et al., 1991), hippocampal (Navarro et al., 2008) and cholinergic basal forebrain (Sarter and Bruno, 2004) areas being most susceptible to age-related alterations.

At a cellular level, the most influential theories used to describe brain aging are the "free radical theory of aging" (Harman, 1956;Harman, 2006;Beckman and Ames, 1998) and "the mitochondrial theory of aging". (Miquel, 1998), both of which place mitochondrial dysfunction as the main cause of age-related metabolic and functional alterations (Melov, 2004;Navarro and Boveris, 2007). "The nitric oxide theory of aging" represents the same line of thinking and states that excessive production of particularly NO is relevant to age-related impairments (McCann et al., 2005). Nevertheless, the cause of brain aging is far from being understood.

Mitochondrial dysfunction increases levels of reactive oxygen species (ROS), impairs intracellular calcium homeostasis and leads to problems in ATP production. Selective age-related decreases of the brain mitochondrial electron transport chain are thought to render the aged brain mitochondria unable to respond to an increased ATP demand (Joo et al., 1999;Toescu, 2005;Navarro and Boveris, 2007). Terms such as decreased homeostatic reserve (Toescu, 2005) or increased allostatic load (McEwen, 2000) have been used to describe the aged neurons. They seem to be able to maintain adequate function during normal activity but are unable to cope with increased metabolic demand, excitatory activation or cellular stress.

Whereas age-related changes in neuronal function have been studied excessively, much less in known about corresponding functional changes in astrocytes (Cotrina and Nedergaard, 2002). Most recent studies report reduced neuroprotection by aged astrocytes (Garcia-Matas et al., 2008). Proteomic studies also show changes in enzymes related to glycolytic production of energy, such as an age-related increase in lactate dehydrogenase expression (Poon et al., 2006; Pawlyk et al., 2007).

1.4. Sleep and brain energy metabolism

1.4.1. Brain energy metabolism across vigilance states

In general, states with increased cortical arousal (waking and REM sleep) are associated with a high metabolic rate, while SWS is associated with a marked reduction of the whole brain's energy status. Human studies show consistent results with various parameters: glucose utilization, oxygen consumption and cerebral blood flow during the normal sleep-wake cycle (Buchsbaum et al., 1989;Madsen et al., 1991;Maquet et al., 1990). In animals, glucose (Kennedy et al., 1982;Ramm and Frost, 1986;Netchiporouk et al., 2001), ATP (Reich et al., 1972) and glycogen (Karnovsky et al., 1983) have been reported to decrease during wakefulness as compared to SWS, whereas levels of adenosine and lactate increase (Porkka-Heiskanen et al., 1997; Richter and Dawson, 1948; Reich et al., 1972;Shram et al., 2002;Kalinchuk et al., 2003).

However, these metabolic variations are not uniform across brain areas. The regional blood flow and metabolic rate are lowest in the frontal areas during SWS and positively correlate with EEG SWA increase (Buchsbaum et al., 1989;Maquet et al., 1990;Braun et al., 1997;Hofle et al., 1997;Dang-Vu et al., 2005). The frontal areas, as well as the cholinergic basal forebrain, are also more likely to show signs of increased metabolic load and sleep pressure early in the course of sleep deprivation (Finelli et al., 2000;Cajochen et al., 1999;Tinguely et al., 2006;Drummond et al., 2000;Urrila et al., 2004;Porkka-Heiskanen et al., 1997;Kalinchuk et al., 2003). These data suggest that regions which exhibit the highest relative activity during wakefulness are also more sensitive to the effects of sleep deprivation and demonstrate that both brain energy metabolism and sleep are regulated in a use-dependent manner.

1.4.2. Glycogen and sleep

After the initial formulation of the hypothesis by Benington and Heller, which states that replenishment of brain glycogen stores is the function of sleep, several studies have been performed to test the hypothesis by directly measuring brain glycogen during sleep and wakefulness, but with confusing results. Some studies found decreases in glycogen or changes in glycogen metabolizing enzymes in brain samples collected after prolonged waking while others

did not (reviewed in Scharf et al., 2008). Changes in glycogen levels seem to be brain-site specific, dependent on strain of animals and the sleep deprivation protocol used. This is not surprising when taking into account that brain energy metabolism is tightly coupled to activation and that brain areas have unique activation patterns related to specific behaviors.

1.4.3. Adenosine and sleep

Several lines of converging evidence now support the role of adenosine as the most important endogenous sleep factor both in humans and animals. Animal studies have demonstrated that extracellular adenosine and its metabolizing enzymes have a diurnal rhythm (Porkka-Heiskanen et al., 2000;Huston et al., 1996;Murillo-Rodriguez et al., 2004;Chagoya, V et al., 1993; Mackiewicz et al., 2003;Alanko et al., 2003a) and that systemic or local administrations of adenosine or its receptor agonists increase sleep and EEG slow wave activity (Feldberg and Sherwood, 1954;Haulica et al., 1973;Radulovacki, 1985;Benington et al., 1995;Ticho and Radulovacki, 1991;Satoh et al., 1996;Schwierin et al., 1996;Portas et al., 1997;Porkka-Heiskanen et al., 1997;Porkka-Heiskanen et al., 2000).

The most detailed description and compelling evidence for adenosine's role as a homeostatic sleep factor came from studies of the cholinergic basal forebrain during prolonged waking (Porkka-Heiskanen et al., 1997;Porkka-Heiskanen et al., 2002;McCarley, 2007). In contrast to other brain areas (Porkka-Heiskanen et al., 2000), increases in extracellular adenosine levels in the BF are very sensitive to even short periods of prolonged waking; adenosine levels continue to rise throughout the waking period, and do not decline until sleep is initiated. Furthermore, the sleep-inducing effect of adenosine is most efficient when adenosine or its agonists are applied locally into the BF, and the compensatory sleep response to sleep deprivation is significantly reduced when the increase in adenosine or A_1- receptor activation is blocked (Kalinchuk et al., 2006a;Thakkar et al., 2003a;Kalinchuk et al., 2008).

As the BF is essential for wake-promotion, the sleep-inducing effect is thought to be mediated via inhibition of the wake active BF cells (Alam et al., 1999;Thakkar et al., 2003b;Arrigoni et al., 2006). In the adjacent VLPO, adenosine excites or disinhibits the sleep promoting neurons (Chamberlin et al., 2003;Morairty et al., 2004;Gallopin et al., 2005;Methippara et al., 2005) thus further enhancing the probability of sleep. In the leptomeningeal area surrounding the BF, the

somnogenic effect of prostaglandin D2 was shown to be mediated via adenosine and A_{2A} –receptors (Satoh et al., 1996;Huang et al., 2007). Adenosine may also act by reducing the orexinergic and histaminergic tone during wakefulness (Thakkar et al., 2002;Thakkar et al., 2008;Hong et al., 2005). Finally, adenosine contributes to disfacilitation in the cortex by reducing the activating input of arousal systems (cholinergic, monoaminergic) and thus making the conditions favorable for SWA (Timofeev et al., 2001;Steriade and McCarley, 2005).

In humans, functional polymorphism in the adenosine deaminase (ADA) gene contributes to the high inter-individual variation in SWS duration and intensity (Retey et al., 2005). Individuals carrying an allele that reduces ADA activity, leading to increased adenosine levels, have longer and deeper SWS episodes. During sleep deprivation A_1–receptor binding increases in human brain (Elmenhorst et al., 2007). Another line of evidence supporting adenosine's role as a mediator of sleep pressure comes from caffeine –a competitive A_1- and A_{2A}–receptor antagonist and the most widely used stimulant in the world (Fredholm et al., 2005b). Caffeine reduces the build-up of sleepiness-related increase in the waking EEG theta activity, it increases sleep latency and reduces SWS intensity (Landolt et al., 2004;Landolt et al., 1995;Schwierin et al., 1996) thus mimicking the changes in the sleep EEG that are known to be associated with reduced homeostatic sleep pressure (Landolt, 2008). Studies on knock-out mice suggest that the wake-promoting effect of caffeine is mediated via the A_{2A}–receptors (Huang et al., 2005) and in humans distinct A_{2A}-receptor gene polymorphism modulates individual caffeine sensitivity (Retey et al., 2007). Caffeine sensitive individuals also seem to be more prone to attentional impairments during sleep deprivation.

1.4.4. Nitric oxide and sleep

Several studies demonstrate that also NO is involved in sleep-wake regulation (Gautier-Sauvigne et al., 2005). Brain NO levels fluctuate in a state-dependent manner (Burlet and Cespuglio, 1997;Vincent et al., 1998) and systemic administration of NOS inhibitors have been shown to decrease sleep (Kapas et al., 1994;Dzoljic et al., 1996;Monti et al., 1999; Monti et al., 2001;Ribeiro et al., 2000;Ribeiro and Kapas, 2005;Cavas and Navarro, 2006) whilst NO donors increase sleep (Kapas and Krueger, 1996;Monti and Jantos, 2004a). Knock-out mice with inactive nNOS or iNOS were reported to have less REM sleep (Chen et al., 2003).

Manipulating NO levels locally in different brain areas during the spontaneous sleep wake cycle, however, has provided somewhat confusing results. Inhibition of NO synthesis in the cholinergic LDT/PPT area and in the noradrenergic raphe nuclei decreased sleep or decreased only REM sleep (Datta et al., 1997;Leonard and Lydic, 1995;Hars, 1999;Monti et al., 1999;Monti et al., 2001). Decreasing the level of NO in the basal forebrain decreased SWS and increased waking (Monti and Jantos, 2004b) but had no effect in another study (Vazquez et al., 2002). No effect was found when the NO level was increased (Monti and Jantos, 2004b).

Recent studies investigating the role of NO in homeostatic sleep regulation revealed a specific mechanism by which NO is involved in the generation of sleep pressure. It was found that NO enhances adenosine release in the BF during sleep deprivation (Kalinchuk et al., 2006a;Kalinchuk et al., 2006b;Kostin et al., 2008). Similar to adenosine, NO levels were found to increase site-specifically in the BF during SD. Blocking of NO synthesis reduced both adenosine release and the subsequent recovery sleep response, while an infusion of a NO donor stimulated adenosine release and promoted sleep (Kalinchuk et al., 2006a). Surprisingly, it was found that NO release during SD is mediated by the activation of the inducible NOS, the transcriptionally regulated isoform which is not normally present in the brain tissue. Based on this finding, it was suggested that iNOS-mediated NO production is one of the key mechanisms that leads to adenosine release in the BF during prolonged waking and thus contributes to the homeostatic increase in SWS during recovery sleep.

1.5. Basal forebrain and sleep-wake regulation

1.5.1. BF regulation of cortical arousal

The BF is a heterogeneous brain region located close to the preoptic and supraoptic areas of the hypothalamus. The definition of the BF nuclei differs in the literature but usually includes the horizontal diagonal band (HDB), substantia innominata (SI), magnocellular preoptic nucleus (MCPO), medial septum (MS), vertical limb of the diagonal band (VDB) and the nucleus basalis magnocellularis (NBM) The BF nuclei contain cortically projecting cholinergic, GABAergic and glutamatergic cells that are essential in the regulation of cortical arousal and attention (Mesulam et al., 1983;Szymusiak, 1995;Szymusiak et al., 2000;Semba, 2000;Jones, 2004;Gritti et al., 2006;Jones, 2008;Parikh and Sarter, 2008). In addition, the cholinergic cells have been implicated in cortical plasticity (Hasselmo, 2006;Hasselmo and Giocomo, 2006).

Cells in the BF have wide projections to the cortex with particularly dense input to the prefrontal cortex and also provide the main cholinergic input to the cortical mantle (Henny and Jones, 2008). In rodents the primary source of neocortical cholinergic inputs stems from the HDB as well as the ventral NBM and SI (Mesulam et al., 1983;Armstrong et al., 1983;Luiten et al., 1987). BF cells also contact the reticular thalamic nucleus, amygdala, hypothalamus, hippocampus and the brainstem (Gritti et al., 1997;Zaborszky et al., 1999). Ascending input to the BF is received from brainstem arousal systems (Semba et al., 1988) whereas the descending input comes primarily from the prefrontal cortex (Zaborszky et al., 1997;Golmayo et al., 2003). Recordings of BF neurons have shown that most cells in the BF are active during EEG activation (wakefulness and REM sleep) and increase their firing in association with EEG theta and gamma oscillations (Detari et al., 1999;Szymusiak et al., 2000;Manns et al., 2000). Glutamatergic activation of the BF cells has been shown to increase cortical ACh release, EEG activation (theta and gamma oscillations), induce waking and suppress sleep (Lamour et al., 1986;Manfridi et al., 1999;Cape and Jones, 2000;Fournier et al., 2004) whereas inactivation of the BF or BF lesions reduce cortical arousal (Buzsaki et al., 1988b;Vanderwolf et al., 1993) and impair attentional performance (Sarter and Bruno, 1997). Activation of the BF cholinergic cells *in vitro* with the glutamate receptor agonist NMDA induced burst firing, and this mode may be the mechanism by which the BF facilitates cortical oscillations (Khateb et al., 1992;Khateb et al., 1995). Recent measurements of cortical ACh release with microelectrodes indicate that ACh acts on multiple time scales: slowly in a minute time-scale to control arousal, and transiently in a sub-second time-scale to control cue-detection (Parikh et al., 2007).

1.5.2. BF and aging

As cholinergic dysfunction leads to a wide range of cognitive impairments, it has been suggested that alterations in BF function may contribute to the decline in cortical functions associated with aging (Sarter and Bruno, 2004;Schliebs and Arendt, 2006). In contrast to brain pathological states, such as Alzheimer's, healthy aging is not associated with significant loss of cholinergic neurons, but rather with morphological and functional alternations (Armstrong et al., 1993;Sarter and Bruno, 2004;Murchison and Griffith, 2007). Several studies have also shown age-related attenuation in cortical ACh release, particularly during exploratory behavior, presentation of

novel stimuli or experimental BF activation (Mitsushima et al., 1996;Giovannini et al., 1998;Fadel et al., 1999;Herzog et al., 2003).

The reason for the selective sensitivity of the BF to aging is not known. *In vitro* studies comparing responses of cholinergic neurons to nitrosative or oxidative stress have found that the BF neurons are remarkably more vulnerable to these stressors than the brainstem cholinergic neurons (Personett et al., 2000;McKinney et al., 2004;McKinney and Jacksonville, 2005). The cholinergic cells differ from other neuronal cell types by utilizing acetyl-CoA not only for energy production but also for acetylcholine synthesis, which has been suggested to reduce their capacity for energy production. Cholinergic cells appear to have higher energy demand, and a recent microarray study found that the age-dependent increase in expression of genes involved in energy production was more pronounced in the BF cholinergic population as compared to brain stem cholinergic neurons (Baskerville et al., 2008). These data implicate that even at baseline aged BF cholinergic cells may suffer from energy shortage.

1.5.3. BF and sleep-wake homeostasis

The site-specific accumulation of the sleep inducing adenosine in the BF during prolonged waking and the fact that the effects of adenosine are primarily mediated via the BF provide solid evidence for the importance of the BF in homeostatic sleep regulation (reviewed in:(Porkka-Heiskanen et al., 2002;Basheer et al., 2004;McCarley, 2007). The increase in the extracellular adenosine level is accompanied by selective increases also in the extracellular levels of the energy metabolites lactate and pyruvate as well as nitric oxide (Kalinchuk et al., 2003;Kalinchuk et al., 2006a;Kalinchuk et al., 2006b). Based on this evidence it is clear that the BF is selectively sensitive to the effects of prolonged waking. It has been suggested the BF could function as “an early response center” of the sleep homeostasis coupling energy demand to sleep promotion. However, the exact cellular mechanisms explaining the increased sensitivity of the BF to SD are not known.

Increases in extracellular adenosine levels could possibly result from: 1) inhibition of enzymes that mediate adenosine breakdown (adenosine kinase, adenosine deaminase or SAH hydrolase) 2) decreased transporter activity because in baseline inward flux of adenosine dominates 3) increased extracellular formation of adenosine from ATP or 4) increased intracellular ATP

breakdown/energy shortage. Experimental evidence in rats has shown that sleep deprivation in rats does not affect the activity of adenosine kinase, ecto- and endonucleotidases or adenosine deaminase (Alanko et al., 2003a;Mackiewicz et al., 2003)

The decreased substrate binding of adenosine transporter ENT1, that was found, may contribute to increased adenosine levels, as pharmacological blocking of the ENT1 is known to increase extracellular adenosine levels (Alanko et al., 2003b). As the inhibition of extracellular AMP hydrolysis does not increase extracellular adenosine levels (Rosenberg et al., 2000), the most likely explanation of increased extracellular BF adenosine is an intracellular formation of adenosine resulting from increased ATP consumption or energy shortage.

Both neuronal activation (Modirrousta et al., 2004) and energy demand increase in the BF during prolonged waking. In favor of the hypothesis that adenosine production results from energy shortage it was demonstrated that increased BF extracellular adenosine, lactate and pyruvate levels, as well as sleep promotion, could be induced in an animal model of local energy depletion (Kalinchuk et al., 2003). Furthermore, recent findings imply that induction of the iNOS and production of NO are required for adenosine production in the BF during prolonged waking (Kalinchuk et al., 2006a). As NO is known to inhibit oxidative phosphorylation (see section 1.3.6.) it may further potentiate the decrease in the cellular energy charge by reducing ATP production and thus leading to adenosine increase. Nevertheless it is not known whether changes in the cellular energy charge take place in the BF during prolonged waking and what type of neuronal activation is needed for such effects to occur.

Recent data strongly suggest that the activity of the cholinergic BF cells plays a major role in the development of sleep pressure during prolonged waking as selective lesioning of the BF cholinergic cells with the immunotoxin saporin 1) abolishes the SD induced increase in extracellular adenosine levels (Blanco-Centurion et al., 2006), 2) attenuates waking EEG theta activity (Kaur et al., 2008), and 3) reduces the homeostatic recovery sleep response (Kalinchuk et al., 2008).

As aging is associated with cholinergic dysfunction and changes in the energy status of the BF cholinergic cells (see section 1.5.2.), the aged BF is a powerful model whose study could provide deeper insight into the cellular mechanism contributing to the generation of sleep pressure.

2. AIMS AND HYPOTHESES OF THE STUDY

This thesis was aimed at further understanding the processes by which sleep pressure is generated during prolonged waking, and particularly the role played by the cholinergic basal forebrain (BF) was investigated.

2.1. Aims and the study system

Two major questions were asked:

- Which cellular events in the BF lead to increases in extracellular adenosine and lactate levels during prolonged waking and contribute to the generation homeostatic sleep pressure?

- Could age-related alterations of the BF function during prolonged waking contribute to the age-related changes in homeostatic sleep-wake regulation?

The role of BF in sleep-wake regulation was studied simultaneously at three levels of organization:

1 Locally at a cellular level by measuring energy metabolites and expression of proteins in the BF.

2 Globally at a cortical level (the out-put area of the BF) by measuring EEG oscillations.

3 At a behavioral level by studying changes in vigilance states and manipulating behavior by sleep deprivation.

See figures 2.1.1 and 2.1.2 for detailed description of the study system. This system provided the framework for the working model of the present thesis.

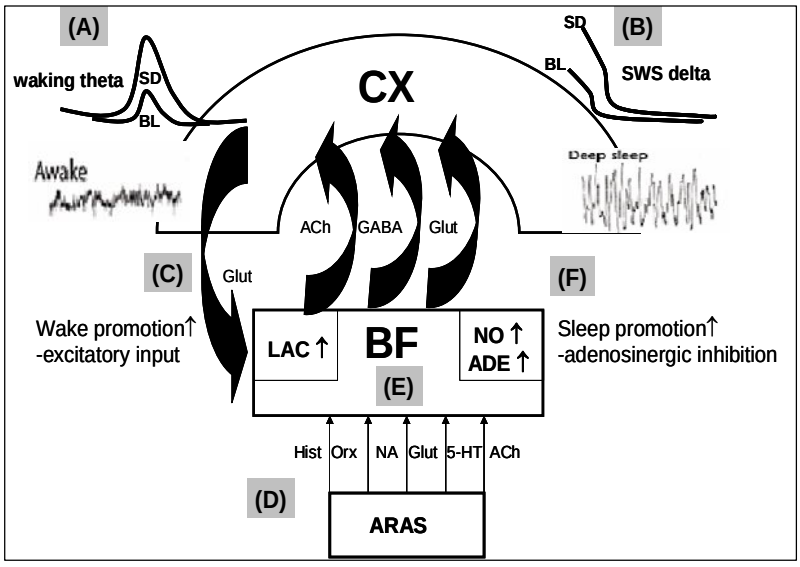


Fig. 2.1.1. Mechanisms of BF sleep-wake regulation (global). **(A)** During prolonged waking/sleep deprivation (SD), EEG theta power is increased as compared to baseline (BL). **(B)** During the subsequent recovery sleep, SWS delta power is increased as compared to BL.
The cortically projecting cells in the basal forebrain (BF) are critically involved in regulation of cortical (CX) arousal: inactivation of the BF induces sleep and SWS delta activity, while its activation promotes wakefulness and fast EEG rhythms (such as theta). **(C, D)** During prolonged waking BF is challenged by constant or increased neuronal activation by the cortex and by the ascending arousal systems (ARAS)**. (E)** Extracellular nitric oxide (NO), adenosine and lactate levels increase. **(F)** Increased adenosine levels lead to increased adenosinergic inhibition of the BF and thus promotion of sleep and SWS delta. (ACh=acetylcholine, GABA=gamma-aminobutyric acid, Glut= glutamate, Hist= histamine, Orx=orexin, 5-HT=serotonin).

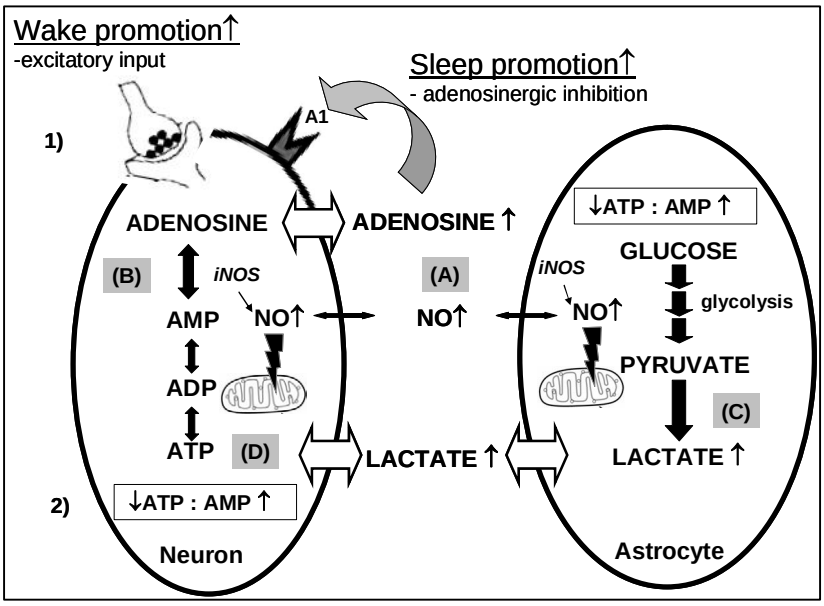


Fig. 2.1.2. Mechanisms of BF sleep-wake regulation (local). **(A)** NO inhibits mitochondrial energy production. **(B)** Exracellular adenosine levels increase when the cellular energy charge (ATP↓AMP↑) decreases. **(C)** Lactate release is coupled to neuronal activity. Lactate production results from glycolytic break-down of glucose, mostly in astrocytes. **(D)** Lactate is used in neurons as an energy fuel during increased neuronal activity**.**

One of the aims of the present thesis was to investigate whether adenosine and lactate release result from **1)** increased excitatory input and/or **2)** decreased cellular energy charge.

2.2. Working model and hypotheses

The main hypothesis and the working model are presented in figure 2.2.1.

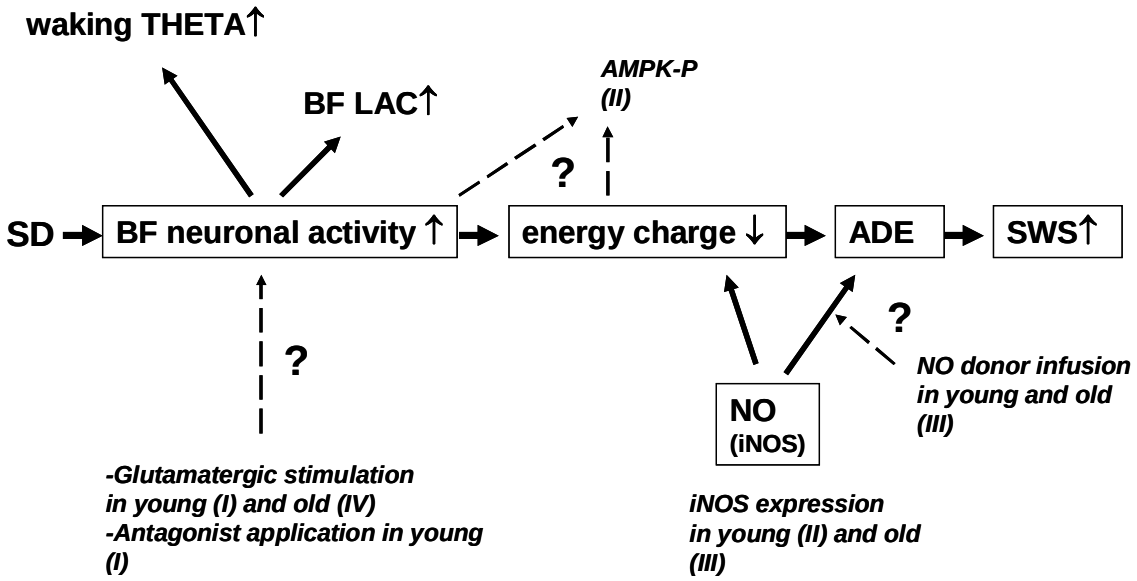


Figure 2.2.1.Working model: Sleep deprivation (SD) increases neuronal activity in the BF, which increases waking EEG theta power and BF lactate release. Sustained BF neuronal activity decreases the cellular energy charge (AMP levels increase and ATP decrease). This alone or together with the neuronal activity-mediated increase in intracellular calcium activate the cellular energy sensor AMPK. The iNOS mediated NO production further inhibits ATP production and increases extracellular adenosine (ADE). Adenosinergic inhibition of the BF promotes SWS/recovery sleep. The present thesis was aimed at testing this model. The specific hypothesis and manipulations to test this model are shown in *italics*, the specific studies/original publications are referred by their Roman numerals, presented with dashed arrows and described below.

Specific hypothesis of the studies were:

I. Similarly to SD, experimental neuronal activation of the BF would elevate extracellular adenosine and lactate levels in the BF and increase the subsequent sleep. Decreasing BF activation during SD would decrease the subsequent recovery sleep.

II. AMPK- a marker of decreased cellular energy charge is activated in the BF during SD.

III. In aging, NO (and adenosine) mediated sleep promotion is attenuated.

IV. BF neuronal activity and the following lactate release are needed to sustain active waking (waking theta) during SD. In aging this mechanism is impaired.

3. MATERIALS AND METHODS

Details of the materials and methods are given in the original publications (I-IV).

3.1. Animals

All animal procedures were approved by the University of Helsinki Ethical Committee for Animal Experiments and by the Regional Committee of the State Provincial Office and performed according to applicable national and EU legislation. All efforts were made to minimize the number of animals used and their suffering.

Three age groups of male HanWistar rats: young (Y, 3-4 months, 300-400 g) middle-aged (M, 12-14 months, 400-500 g) and old (O, 23-25 months, 500-600 g) were housed under a 12 h light/dark cycle (lights on at 8:30 AM) at constant temperature (+22±1 °C). Food and water were provided *ad libitum* and the animals were gradually habituated to handling at least 1 week before experimental procedures. EEG recording and *in vivo* microdialysis was used in studies I, III and IV, while in study II intact animals were decapitated following 3-6 h of prolonged waking/sleep deprivation (SD) and brains sampled for protein analysis. Rats without chronical EEG/EMG electrodes were group-housed while rats with electrodes were single-housed after surgery.

3.1.1. Sleep deprivation (SD)

To increase homeostatic sleep pressure and to induce subsequent recovery sleep rats were sleep deprived. Whenever rats appeared to be sleepy or slow waves were visible in the EEG, new objects such as wooden blocks, new bedding material and stretchable strings were introduced and/or the cage was gently tapped. This method is referred to as sleep deprivation (SD) by gentle handling and it has been shown to stimulate active waking (Huber et al., 2007). In all of the studies with chronic EEG/EMG and microdialysis, rats were sleep deprived only for 3 hours (I, III, IV).

3.1.2. Surgery

Under general barbiturate or ketamine anesthesia (dosages can be found in the original article) rats were placed in a stereotaxic instrument and implanted with:

1) Chronic epidural **EEG electrodes** (gold plated miniature dental screws) placed fronto-parietally to the skull.
2) Nuchal electromyogram **(EMG) electrodes** (Teflon coated thin silver wires).

-Both for the assessment of vigilance states and EEG activity.

3) **Microdialysis guide cannula** (CMA 11 guide, CMA/Microdialysis) for subsequent insertion of the microdialysis probe. The guide cannula was inserted unilaterally 2 mm above the basal forebrain area.

The guide cannula and the electrode connector were fixed to the skull with acrylic dental cement. After the operation animals were single-housed and allowed to recover from surgery from 1 to 2.5 weeks depending on age.

3.2. Recording of EEG and EMG

Animals were connected to flexible counter-balanced cables for EEG/EMG recording and habituated to recording cables for 2-3 days. The experiments were not started until behavior and sleep-wake cycle had normalized. EEG and EMG were recorded to monitor vigilance states and EEG markers of sleep during the experiments. The EEG/EMG signal was fed to a 8-channel digital amplifier (Cyber Amp 380, Axon Instruments); amplified (gain 10 000); analogically filtered (high pass: 0.1 Hz, low pass 40 Hz), digitized at 104 Hz with a 1401 unit (CED, Cambridge Electronic Devices), monitored on-line during the experiments and stored using Spike2 software (CED) for further off-line analysis.

3.2.1. Vigilance state scoring

For monitoring of the sleep-wake behavior during the habituation period and to determine the overall amount and distribution of sleep during the experimental days, we used semi-automatic scoring to detect SWS. Semi-automatic scoring was performed with a self-made script in Spike2 in 30s epochs (described and validated in (Stenberg et al., 2003;Kalinchuk et al., 2003). The presence and distribution of REM sleep was checked manually. For manual scoring the EEG was analyzed in 4 s or 5 s epochs using Spike2 sleep scoring script Sleepscore v1.01 (CED, Ltd.). Vigilance states (wake, SWS, REM) were scored according to standard criteria. Artifacts or epochs with mixed states were marked and excluded from further analysis.

3.2.2. Spectral analysis

Mean EEG power (μV^2) spectra were generated in Spike2 (CED, Ltd.) separately for consecutive wake epochs and SWS epochs (fast Fourier transform, FFT = 256, Hanning window, 0.4 Hz resolution) within a frequency range of 0.8- 25 Hz. Absolute power values were always

normalized to total power of the individual recordings before further analysis. All power values are shown as relative power: power/number of epochs used to generate spectra. Waking-theta power was calculated from the power spectra within the frequency range of 4.9-9.3 Hz, SWS delta power within the frequency range 0.8-4 Hz.

3.3. *In vivo* microdialysis

Brain microdialysis is a technique for collection of molecules in the extracellular fluid by means of a semipermeable membrane at the tip of microdialysis probe. When the probe is slowly perfused with a continuous flow of artificial cerebrospinal fluid (aCSF), passive diffusion of molecules according to their concentration gradient takes place across the membrane. The strength of the method is that it allows simultaneous application and monitoring of the effects of drugs in a restricted brain area. Furthermore, as it can be performed in freely moving animals, information on changes in the composition of the extracellular fluid can be continuously monitored across vigilance states or during experimental manipulations. Application of microdialysis was one of the key factors leading to the establishment of adenosine as an endogenous sleep factor in the BF (see sections 1.4.3. and 1.5.3. and (Basheer et al., 2004). In the present studies we used *in vivo* microdialysis to collect samples in the BF for quantitative analysis of adenosine, lactate and NO. In addition, microdialysis was utilized to locally deliver glutamate or its agonists (AMPA, NMDA), antagonist (MK-801) or uptake-blocker (DHK) or NO donor (DETA/NO) into the BF (details of drugs, concentrations and suppliers can be found in the original articles).

3.3.1. Sample collection and experimental schedule

Twenty hours before the first experiment, microdialysis probes (CMA 11 probe, CMA/Microdialysis) were lowered into the BF. On the day of the experiments between 8:30 and 9:30 AM animals were connected to microdialysis tubing as well as to the EEG/EMG recording cables. Tubing was attached to a microdialysis syringe pump (CMA/Microdialysis) for continuous perfusion (1 µl/min) of aCF. For each experiment, collection of 30 min (30µl) samples was started at 10:00 AM and continued for 6 hours until 4:00 PM (altogether 12 samples were collected). At the end of the microdialysis period animals were disconnected from the microdialysis tubing while the EEG/EMG recording was continued until the next morning (8:30 AM).

During the first experimental day (baseline day) animals were left undisturbed (except for connecting/disconnecting of microdialysis tubing in the beginning and end of the microdialysis period) to allow spontaneous sleep-wake behavior to occur. On the following days before experimental procedures aCSF perfusion alone was carried out for 2-3 hours to collect samples for the determination of the pre-treatment levels of metabolites. The following experiments were performed:

1) sleep deprivation (SD) with sample collection only (I, III, IV)
2) glutamatergic activation (AMPA, NMDA, DHK, L-glutamate) of the BF (I, IV)
3) NO donor (DETA/NO) infusion (III)
4) sleep deprivation with simultaneous glutamate receptor antagonist (MK-801) infusion (I)

SD was always performed first and a minimum of 48h was allowed between experiments.

3.3.2. Verification of probe location

After the end of all the experiments animals received a lethal dose of pentobarbital. Color ink was injected through a modified microdialysis probe to mark the location of the probe tip. The brains were removed and frozen on dry ice. 20 µm coronal frozen sections were cut; stained with Toluidine Blue; dehydrated through graded ethanol, cover-slipped with Depex and visually inspected under a light microscope to verify the location of the probe tip using a rat brain atlas (Paxinos and Watson, 1998). Only those animals with probe locations in a close vicinity of the target area (BF) including horizontal diagonal band of Broca (HDB), substantia innominata (SI), magnocellular preoptic area (MCPO), lateral preoptic area (LPO) and the basal nucleus of Meynert (B) were included in the analysis.

3.3.3. Determination of neuronal activity with FOS staining

To assure that glutamatergic stimulation of the BF increased neuronal activity, we used FOS staining, which is generally used as a marker of neuronal activity (Sagar et al., 1988). Immediately after 3 h of treatment the animals received a lethal dose of pentobarbital and were under deep anesthesia transcardially perfused with saline followed by 4 % paraformaldehyde in phosphate buffered saline (PBS) after which brains were submerged in 30% sucrose at 4°C. Coronal sections were cut through the BF area and endogenous peroxidase activity blocked with

hydrogen peroxide. Sections were then treated with blocking solution (4 % donkey serum; 0.2 % Triton-X in PBS); incubated overnight with a rabbit anti-FOS primary antiserum (Ab-5, 1:10 000, Calbiochem) and further processed with biotinylated donkey anti-rabbit IgG (1:400; Chemicon International) followed by reaction with avidin-bioting complex (ABC, Vector Elite Kit). Nickel-diaminobenzidine tetrahydrochloride (Ni-DAB; peroxidase substrate kit DAB, Vector laboratories) was used for detection. Sections were mounted on slides and investigated under a light microscope. There was no nuclear staining in the absence of primary antiserum. As reported earlier for NMDA and AMPA (Cape and Jones, 2000), also application of glutamate induced FOS expression. Control treatment with aCSF did not result in specific FOS staining.

3.3.4. HPLC analysis of adenosine and lactate

Microdialysis samples were analyzed for adenosine and lactate using high performance liquid chromatography (HPLC) coupled to UV detection. The adenosine concentration was measured as previously described in detail (Porkka-Heiskanen et al., 1997). Lactate was assayed as in (Hallstrom et al., 1989). The detection limits of the assays were for adenosine 0.8 nM (signal-to-noise ratio 2:1), and for lactate 10 µM (signal-to-noise ratio 3:1) (Grob, 1985). Samples with concentrations under the detection limits were discarded from further analysis. All HPLC data were analyzed with CLASS-VP 6.12 software (Shimadzu Corporation) and quantified by comparing the peak areas to those of standards. Microdialysis samples collected during the hours before the treatment served as a baseline for each individual animal. The mean concentration of samples collected either during the treatment or during the final hour of aCSF perfusion was compared to this baseline for each animal.

3.3.5. Nitric oxide detection

As no endogenous source other than NO is known for nitrite (NO_2^-) and nitrate (NO_3^-) (collectively NO_x^-) in the brain, this metabolite has generally been taken as indicative of NO production (Mackenzie et al., 1996). The dietary intake of nitrite can considerably affect the plasma nitrate concentrations, but as these anions do not penetrate the blood-brain barrier, their effect on brain concentrations is not significant (Clark et al., 1996). The diffusion range of NO is ~400 µm determined from brain slices (Ledo et al., 2005). Concentrations of NO_x^- were measured from microdialysis samples using a Nitrate/Nitrite Fluorometric Assay kit (Cayman Chemical Company).

3.4. Western blot

Rats were divided into two groups: the SD group and their circadian controls. SD started at lights on and rats were sacrificed by decapitation following 1.5-, 3- or 6 hours of SD. Control rats were left undisturbed and sacrificed at the same time points.

After decapitation, the brains were immediately removed and the BF and cortex were quickly dissected, snap-frozen in liquid nitrogen and stored at -80°C. Frozen tissues were homogenized in a 1:3 ratio (g tissue/ ml buffer) to ice cold homogenization buffer (50mM TRIS-HCl, pH 7.5, 130mM NaCl, 1mM EDTA, 1mM EGTA, 1% (v/v) TX100, 2.5mM Sodium pyrophosphate, 1mM beta-Glyserol-2-phosphate, 1mM phenylmethylsulphonyl fluoride (PMSF)) including inhibitor cocktails (Protease Inhibitor Cocktail, cat# P834 , phosphatase inhibitor cocktail 1. and 2. cat# P2850 and P5726 from Sigma-Aldrich Co, St Louis, MO). After adding 5µl of PMSF (10mM) samples were left to stand for 30min on ice and then centrifuged for 30min at 16000*g* at 4ºC. The resulting supernatants were used for Western blot. The protein concentration was measured with Protein Assay Dye Reagent (Bio-Rad Laboratories Inc.).

Samples were mixed with a 1:1 ratio with Laemmli buffer (Bio-Rad) containing 5% 2-mercaptoethanol (Sigma-Aldrich), denatured by boiling and loaded into a 7.5% sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE). Proteins were resolved by electrophoresis and transferred to Hybond^{TM} –ECL^{TM} nitrocellulose membranes (Amersham Biosciences, UK). High Range Rainbow Molecular Marker (GE Healthcare) was used as a size marker. Membranes were blocked with 5% non fat dry milk in TRIS-buffered saline, pH 7.5 with 0.1% Tween (TBS-T) before incubation with antibodies

3.4.1. Detection of iNOS induction

iNOS protein was measured as in (Kalinchuk et al., 2006a). Shortly, samples were resolved with 10% SDS-PAGE, transferred to nitrocellulose membranes, incubated with polyclonal anti-iNOS primary antibody (1:1000 dilution; at +4º C o/n Santa Cruz, California, US) and secondary FITC-conjugated antibody (1:200 dilution; Zymed Laboratories Inc., San Francisco, US;). *β*-Tubulin, detected with a combination of primary antibody (0.25 mg/ml; Zymed Laboratories Inc., San Francisco, US) and secondary TRITC-conjugated antibody (1:1000; Jackson ImmunoResearch Laboratories Inc., Baltimore, US) was used as a loading control. Mouse macrophage lysate (BD

Biosciences) served as a positive control. Band optical densities were measured with the Typhoon™ variable mode imager and ImageQuant™.

3.4.2. Detection of AMPK activation

AMPK activation was detected with an antibody specific for phosphotreonine 172 within the catalytic α subunits (Phospho-AMPK-α (Thr172) from Cell Signaling Technology Inc., Danvers, MA). Membranes were incubated with a 1:800 dilution of the antibody in TBS-T with 5% bovine serum albumin at +4ºC overnight (o/n). After incubation with the secondary antibody (1:2000, anti-rabbit IgG conjugated to horseradish peroxidase, Cell Signaling) blots were developed using enhanced chemiluminescence (Amersham ECL Advance Western Blotting Detection Kit, GE Healthcare). The ECL signal was detected and quantified with Typhoon™ variable mode imager and ImageQuant™ software (both from GE Healthcare). Total AMPK was measured from the same blots after incubation with stripping buffer (63nM TRIS-HCl, pH 7.6, 100mM 2-mercaptoethanol, 2% SDS) for 30min at +50ºC. Total AMPK detection was similar to pAMPK except that the antibody for AMPK-α subunits (1:1000 dilution, +4ºC, o/n, Cell Signaling) was used as the primary antibody. AMPK Control Cell Extracts (Cell Signaling) and self-made positive controls (described in detail in original publication II) were used as positive controls in each gel.

3.5. Statistical analyses

For simple comparisons of two groups we used *t*-tests or paired *t*-test depending on the experimental design; e.g. whether comparison was made between two time point measures within the same animals (dependent variables, paired design) or between two different groups of animals (independent variables). For analysis of more than two groups we used One Way -or Two Way ANOVA followed by the Holm-Sidak as post-hoc test or One Way- or Two Way Repeated Measures of ANOVA followed by Holm-Sidak multiple comparison procedure, again depending on whether variables were independent such as different age groups or dependent such as time series analysis within a group. Correlation analysis in study IV was performed with Pearson Product Moment Correlation. In all cases equivalent non-parametric statistical tests were used if the data were not normally distributed. Statistical analyses were carried out either in SPSS 12.0.1

or in Sigma Stat ® (version 3.00 or 3.1) statistical software, both from SPSS Science Software (Chicago, IL, USA).

3.6. Critical considerations

3.6.1. *In vivo* microdialysis and cellular sources of metabolites

While *in vivo* microdialysis is an excellent tool to be applied when simultaneous measurements of neurochemistry and behavior or EEG are needed, it does not provide detailed information on the actual cellular sources of the molecules measured. It measures the net extracellular concentration of molecules, which in turn reflects changes in transport, release or diffusion of molecules from intracellular compartments or from the circulation. In the present studies, while it is assumed, based on extensive literature, that lactate release is primarily of astrocytic origin, this is not experimentally verified.

4. RESULTS AND DISCUSSION

4.1. Glutamatergic stimulation of the BF (I)

To test the hypothesis that adenosine and lactate responses in the BF during SD are due to increased or continuous BF activity, we compared the effects of 3 h SD to the effects of 3 h experimental BF activation.

4.1.1. Effects on behavioral states and EEG arousal

Sleep deprivation for 3 h with presentation of novel objects was effective in keeping the rats awake and resulted in significant increase in high frequency (HF) EEG theta power (7-9 Hz). With experimental stimulation of the BF by glutamate or its receptor agonists, three relevant classes of activation were induced:

1) mild BF activation with glutamate or its uptake blocker (DHK), which did not activate the EEG or induce wakefulness
2) wake-inducing activation by AMPA without EEG theta increase
3) wake-inducing activation by NMDA with EEG theta increase

Times spent awake during different treatments are shown in table 1.

Treatment (3 h, between 12-15)	**% time spent awake**
Baseline (aCSF infusion), n = 17	31.0 ± 1.9
Glutamate (5 mM), n = 5	37.3 ± 5.8
AMPA (0.047mM), n = 6	61.3 ± 6.7 *
NMDA (0.3 mM), n = 7	71.0 ± 7.0 *
SD, n = 9	96.0 ± 1.2*

Table 4.1.1. % of time spent awake during the treatment period.
Data is shown as group mean ± S.E.M. Asterisks denote statistically significant ($P \leq 0.05$) differences between treatments and baseline. Time spent awake during AMPA activation did not differ from that induced by NMDA ($P > 0.05$).

4.1.2. Effects on extracellular adenosine and lactate

Activation of the BF with NMDA and AMPA. As reported previously (Kalinchuk et al., 2003) 3h SD increased extracellular adenosine and lactate levels in the BF. A similar effect with physiological increases in both adenosine and lactate level was found when the BF was stimulated with NMDA. No significant increases were found during stimulation with AMPA, even if the time spent awake with AMPA did not differ from that induced by NMDA (table 1). This was puzzling at first, because it was expected that any activation that induces wakefulness and BF activity, would also increase adenosine and lactate levels.

When trying to solve this problem, we hypothesized that a difference in the type of BF activity (or waking) induced by these two agonists, would explain the differences in metabolite responses. To test this assumption we compared waking EEG power spectra recorded during SD, NMDA and AMPA; and found that only with SD and NMDA high frequency (HF, 7-9 Hz) EEG theta power was significantly increased. We concluded that the type of BF activity that occurs during increased HF EEG theta activity is more potent in increasing adenosine and lactate levels in the BF than wakefulness without HF theta increase.

The most important finding of this part of the study was that it demonstrated a difference in the BF adenosine and lactate responses between HF-theta enriched, active waking (SD and NMDA), and waking without HF-theta increase (AMPA). As adenosine is involved in the generation of homeostatic sleep pressure, our results are in line with another study showing that sleep deprivation enriched with active waking is more effective in increasing homeostatic sleep pressure than sleep deprivation with less active waking (Huber et al., 2007).

Glutamate and DHK infusion. During application of glutamate, extracellular adenosine increased to a much higher level than measured with any other treatments (60 fold as compared to 2-4 fold increase) and lactate levels slightly decreased. Application of the non-transportable astrocyte glutamate blocker DHK, which is known to increase extracellular glutamate levels (Massieu et al., 1995;Seki et al., 1999) , slightly increased adenosine levels but had no effect on lactate release.

The effect of local glutamate infusion on adenosine levels most likely results from a metabolic shock brought up by the extensive astrocytic glutamate uptake during the infusion, which glutamate itself, but not the agonists or DHK will activate (Grewer and Rauen, 2005). Previous studies have shown that energy depletion increases extracellular adenosine to a greater extend than electrical stimulation or physiological neuronal activity does (Lloyd et al., 1993;Brundege and Dunwiddie, 1998;Kalinchuk et al., 2003).

The much smaller effect on adenosine, the lack of effect on lactate release during DHK infusion and the absence of wake promotion by these drugs, further support the assumption that changes in metabolite levels are associated with excessive astrocytic glutamate uptake rather than with significant increases in neuronal activity. Furthermore, as adenosine levels were extremely high only during the first hour of glutamate infusion and significantly decreased by the end of the treatment period, it is probable that the BF cells managed to cope with excessive glutamate load and therefore did not suffer from continuous energy depletion.

4.1.3. Effects on subsequent sleep and SWS delta power

In line with the above described findings that only SD and NMDA increased metabolite levels, EEG delta power – a marker of homeostatic sleep drive- was significantly increased only after SD and NMDA. In contrast, all treatments increased the duration of the subsequent SWS, regardless of whether waking or cortical arousal was induced during the BF stimulation. In several earlier studies the amount and the intensity of sleep have also been found to be disproportionally affected (Tobler and Borbely, 1990;Franken et al., 1991;Opp and Krueger, 1994;Cirelli et al., 2005). It is probable that a minor increase in homeostatic sleep pressure (wakefulness without marked increases in adenosine or EEG theta power (AMPA) or experimentally increased BF adenosine without wakefulness (glutamate, DHK), prolong the SWS duration, while a more profound increase in sleep pressure due to active waking (SD, NMDA) also enhances SWS delta power.

Taken together these findings show that both markers of homeostatic sleep pressure: 1) BF adenosine during waking and 2) EEG delta power during sleep, significantly increase only if an increase in HF theta power (a marker of active wake and increased BF activity) is recorded during wakefulness.

4.1.4. Effects of blocking glutamatergic activity in the BF during SD

To further substantiate the assumption that BF activity, and particularly NMDA activation of the BF, contributes to the build-up of sleep pressure during prolonged waking; we reduced glutamatergic activity in the BF during 3h SD by NMDA receptor antagonist (MK-801) infusion. We found that NMDA blockage: 1) had no effect on time spent awake during SD, but significantly reduced HF EEG theta power and 2) reduced SWS duration, but not delta power during the subsequent recovery sleep.

The fact that NMDA blockage selectively reduced HF theta power is in an agreement with our results (described in section 4.1.1) and previous findings from another laboratory (Cape and Jones, 2000) showing that stimulation of the BF with NMDA significantly increases HF (7-9 Hz)- but not LF (5-7) EEG theta power. While selective to the HF-theta, the magnitude of the theta power reduction by BF NMDA blockage was, however, relatively small. This probably contributes to the result that only SWS duration, but not its intensity, was reduced during subsequent recovery sleep. Taken together these results show that elevation of the glutamatergic activity in the BF increases sleep pressure (NMDA, SD), while reduction of BF glutamatergic activity (MK-801) reduces it.

4.2. iNOS and AMPK in the BF during SD (II)

In the study II we tested the hypothesis that BF is selectively sensitive to the metabolic demands of prolonged waking and that induction of iNOS in the BF is accompanied with AMPK activation. AMPK activation increased in the BF but not in the cortex. When iNOS protein levels were measured from the same samples as AMPK, statistically significant increase in iNOS protein was found in the BF but not in the cortex, confirming previous results (Kalinchuk et al., 2006a).

Activation of AMPK in the BF could have resulted either from decreased cellular energy charge or increased neuronal-activity (Hue and Rider, 2007). As NO is well known to inhibit oxidative energy production (Moncada and Bolanos, 2006) and induce AMPK activation (Almeida et al., 2004), we proposed that iNOS and NO mediated activation of AMPK -a mechanism

characterized to promote glycolysis and lactate release in astrocytes, could be one of the protective mechanisms, which by mobilizing supplemental energy stores, sustains BF activation during periods of prolonged waking.

On the other hand, as neuronal activity increases in the BF during prolonged waking (Modirrousta et al., 2004;Nikonova et al., 2005), Dr Kostin personal communication), AMPK may be activated by neuronal activity-mediated rise in the intracellular Ca^{2+}. AMPK activation due to increased neuronal firing has been shown to increase neuronal inhibition by activating $GABA_B$ receptors (Kuramoto et al., 2007).

It is also possible that both AMPK activation pathways are simultaneously activated. Then the activation of AMPK in the BF might be a protective mechanism which, during prolonged waking sustains neuronal activation by mobilizing supplemental energy stores, and at the same time promotes sleep by increasing inhibition of the wake-active cells. Nevertheless, the current findings only suggest that iNOS induction or neuronal activation leads to AMPK phosphorylation in the BF during prolonged waking, but further studies are needed to establish a causal link between these events and AMPK activation.

4.3. Age-related changes in the BF during SD (III, IV)

In the last two studies age-related changes in the BF responses to sleep deprivation were studied, and the effects of SD were compared to glutamatergic (NMDA) stimulation in study IV and to NO donor (DETA/NO) infusion in study III. In both studies three age groups of rats were used: young (~3 moths), middle-aged (~12 months) and old (~24-months).

4.3.1. Reduction in waking EEG theta power during SD (IV)

In study I we had already demonstrated that stimulation of the BF with NMDA or 3h SD enhances the HF (7-9 Hz) EEG theta, while application of NMDA antagonist during SD selectively attenuates the HF theta in young rats. These results implicate that changes in HF-EEG theta power reflect BF activity. In the study IV we found that the increase in HF theta during SD was reduced in both old and middle-aged rats as compared to young. We hypotesized that this reduction could be due to reduced sensitivity of aged BF to excitatory stimulus and tested this by BF NMDA infusion. We found that NMDA did not significantly increase wakefulness or HF

theta in the aged rats. Accordingly, several studies have demonstrated age-related decreases in NMDA receptor density and alternations in function in the brain (Segovia et al., 2001;Gonzales et al., 1991). These results suggest that promotion of cortical arousal by the BF, as measured by the increase in HF theta, is reduced during SD in aging.

During baseline waking LF theta power was higher in old animals than in the young. This finding is consistent with other studies in both humans (van der Hiele K. et al., 2008) and animals (Colas et al., 2005), which found that LF theta power was increased in aged subjects as compared to the young. Several studies have implicated that increased LF theta power correlates with reduced arousal level (Schacter, 1977;Cajochen et al., 1995;Cajochen et al., 2001;Lorenzo et al., 1995;Lafrance and Dumont, 2000;Aeschbach et al., 1999;Aeschbach et al., 1997;Cajochen et al., 2002;Finelli et al., 2000;Strijkstra et al., 2003; Vyazovskiy and Tobler, 2005). In our study reduced baseline arousal level could decrease the capacity of the old to increase active wake (a shift from low- to high frequency theta enriched waking) during SD.

4.3.2. Reduction in the BF lactate release during SD (IV)

In contrast to young, lactate levels did not increase in the BF during SD in the aged animals (middle-aged and old). This could have resulted either: 1) from reduced sensitivity of the aged BF to excitatory input or 2) from age-related impairments in lactate production.

The first assumption is supported by our finding that even experimental stimulation of the BF with NMDA did not increase lactate release or promote waking or increase cortical arousal in the old (described above). Our results indicate that neuronal activity was not increased by NMDA stimulation in the aged and thus did not activate lactate production. In the present study we did not directly investigate the intracellular mechanisms of lactate production or their possible impairments in aging. Nevertheless, both the reduced sensitivity of the aged BF cells to excitatory input as well as reduced capacity for energy production have been reported or implicated in the literature (Sarter and Bruno, 2004; Mitsushima et al., 1996;Giovannini et al., 1998; Fadel et al., 1999;Herzog et al., 2003;Murchison and Griffith, 2007; Baskerville et al., 2008).

To further substantiate the assumption that lactate release in the BF is linked to active waking and HF-theta, we found a strong positive correlation between BF lactate release and HF EEG theta

power in young animals, which was lost in the aged. Taken together our results show that both measures of BF activation: local lactate release and HF EEG theta are reduced in aging.

4.3.3. Reduction in NO and adenosine responses in the BF during SD (III)

In study III we found that, in contrast to young, 3 h SD did not significantly increase iNOS expression or extracellular levels of NO and adenosine in the BF of old animals.

The reduced NO response with aging is in agreement with previous reports demonstrating attenuations in NO production (and iNOS induction) in aged animals in response to immunological challenge or cellular stress (Canuelo et al., 2007;Siles et al., 2002;Xie et al., 2003). We also found that BF NO levels, as compared to young, were chronically high in the aged rats. Thus an explanation for the lack of NO response in the aged could be that because of the high basal NO production, the capacity for further increases is reduced. It is also possible that due to a ceiling effect, small increases in NO production do not lead to a net increase in extracellular NO level.

When comparing young and old animals we found that, similar to NO, the SD-induced adenosine response was attenuated with aging. Although not established in the present study, a causative link between NO and AD has been previously demonstrated (Rosenberg et al., 2000; Kalinchuk et al., 2006a) and thus the lack of significant increase in BF extracellular adenosine in the old rats is probably due to the attenuated NO response.

Interestingly, a previous study (Murillo-Rodriguez et al., 2004) did not report a decrease in adenosine accumulation during SD in aged rats. This discrepancy might be due to differences in data analysis (comparison of pre-SD and SD values from samples collected at the same day in our study, versus comparison of SD day values to baseline day values in their study). Also, according to their data, the first significant increase in adenosine levels in the aged rats occurred only after 4h of SD, while in the young rats it was detected already after the first hour. It is possible that, had we continued SD for longer than 3h, we might have measured increases in adenosine. Nevertheless, the immediate increase in adenosine and NO during SD, characteristic for young animals, is absent in the aged.

Study IV demonstrated that in aging the sensitivity of the BF to excitatory input is reduced: Activation of the BF with NMDA did not induce lactate release or promote waking or cortical arousal, as measured by HF theta power increase. Furthermore, lactate release was absent or HF theta power reduced in aged animals during SD. Based on this information it is possible that during SD, neurons in the BF are less active in the old than in the young. As the accumulation of adenosine is neuronal-activity dependent (for review see Latini and Pedata, 2001) reduced BF neuronal activity during SD in the aged could contribute to the reduced adenosine accumulation.

4.3.4. Reduced sleep promotion by the BF during SD (III, IV)

In our aged animals SWS delta (0.5-4 Hz) power, a marker of sleep intensity and homeostatic sleep drive (Achermann and Borbely, 2003) was reduced in baseline and its increase in response to SD was attenuated. This finding is in line with majority of other studies (Mendelson and Bergmann, 1999a;Mendelson and Bergmann, 1999b;Mendelson and Bergmann, 2000;Shiromani et al., 2000;Gaudreau et al., 2001;Munch et al., 2004;Landolt and Borbely, 2001; Bonnet and Arand, 2007). In studies III and IV we found clear age-dependent functional impairments of the BF during SD, which may contribute to reduced homeostatic sleep response.

In study III we first found that increases in NO (and adenosine) during SD were attenuated (described in section 4.3.3). Second, the experimental increase in NO (by DETA/NO infusion) in the BF did not increase SWS delta power in the middle-aged or old animals, as it did in the young. We proposed that 1) decreased production of NO (and adenosine) as well as 2) the insensitivity of the BF to sleep promotion by NO (and adenosine) both contribute to the diminished homeostatic sleep response in aging.

In the IV study we show that promotion of active wake and cortical arousal (as measured by HF EEG theta) by the BF during prolonged waking is impaired in aging. This may contribute to the reduced homeostatic sleep response, as in the study I we show that wakefulness with HF theta increase is more effective in inducing sleep than wakefulness without HF theta increase, and that reduction of BF activity during SD reduces recovery sleep. A study by another laboratory (Huber et al., 2005) found that wakefulness with increased levels of active wake is more potent in increasing sleep pressure and sleep delta power than the same amount of quiet waking. Thus the

attenuation of homeostatic sleep response could be due to the fact that aged animals spent less time in active wake.

4.4. Major findings

Major findings are presented in figures 4.4.1 and 4.4.2

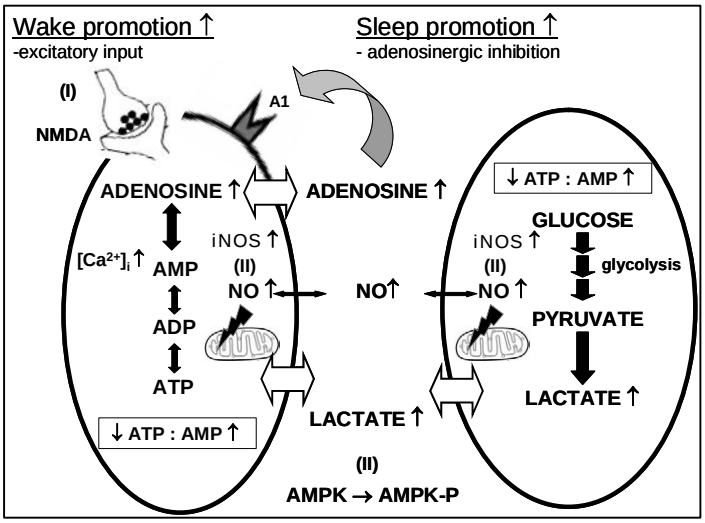


Figure 4.4.1. Major findings-young.
Study I showed that NMDA receptor activation, which increased wakefulness and cortical arousal, also increased adenosine and lactate levels in the BF, and resulted in promotion of sleep subsequently. Study II showed that the site-specific induction of iNOS in the BF is accompanied with AMPK activation (pAMPK).

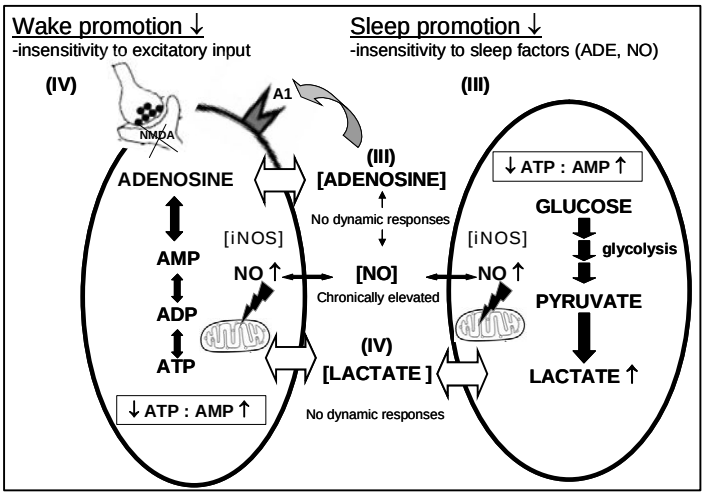


Figure 4.4.2. Major findings-old.
Studies III and IV showed that during SD, increases in cortical arousal as well as adenosine, lactate and NO level in the BF were attenuated. Study IV showed that the BF is insensitive to wake promoting BF stimulation by the glutamate receptor agonist NMDA. Study III showed that the BF is insensitive to NO mediated sleep promotion. Thus in aging both wake promotion and sleep induction via the BF are reduced.

4.5. Future perspectives

First, it would be important to determine the major source of increased excitatory input to BF. Does it arrive from the prefrontal cortex or from the activating arousal systems? Which cells in the BF are most affected by increased activation (cholinergic, GABAergic or glutamatergic)? Preliminary findings from our laboratory indicate that during SD, a specific population of cells in the BF increases its activity. Elevated activity in the BF during SD could be a compensatory response by which the BF cells counteract adenosinergic inhibition and continue to sustain cortical arousal in spite of increased adenosine levels.

Second, studies should be carried to find out whether AMPK activation is up-stream from iNOS mediated inhibition of mitochondrial energy production or due to NMDA mediated increases in $[Ca^{2+}]_i$ or both. Also it would be interesting to know in which cell types AMPK is activated (neurons or glia). The major unanswered question is, however, why iNOS expression is activated first in the BF during SD, and in which cell types this occurs.

Third, most sleep deprivations in the present studies lasted only for 3 h. This short duration was chosen to reduce the confounding effects of stress, and because previous studies had shown that site-specific changes in BF metabolites can be detected with 3h SD. Nevertheless, such a short SD did not really allow analysis of temporal changes in EEG markers, such as theta during SD. It would be extremely interesting to see whether the increase in HF-theta, that we recorded, would be reduced or replaced by increases in LF-theta when deprivation lasts longer than 3h. In a study by (Vyazovskiy and Tobler, 2005) LF waking theta was increased in the final hours of 6 h SD when recorded during quiet waking epochs.

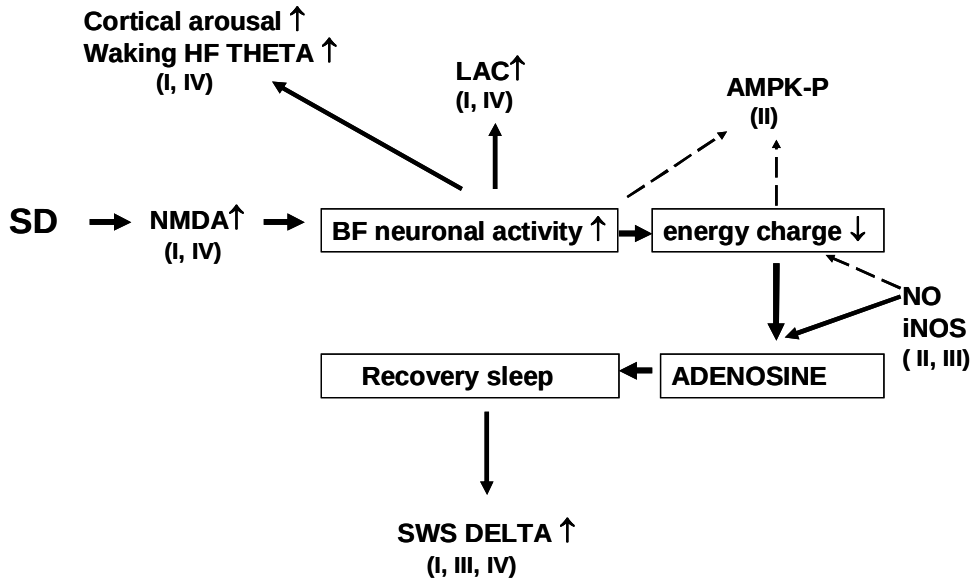
Finally, future studies should determine the causes of age-related BF alterations. Do they result from changes in cellular energy status, calcium homeostasis, or NMDA function? The most interesting question, however, concerns the attenuated homeostatic sleep response in aging. Is it due to reduced capacity of the aged brain to produce sleep (in our study implicated by reduced production and sensitivity of the BF to sleep promoting factors)? If this is true, then it can be assumed that aged individuals live under constant sleep deficit. Another possibility is that sleep need is simply reduced in aging. Our studies also support this possibility, as the accumulation of sleep inducing factors was attenuated in the aged animals and they spent less time in HF-theta

enriched active waking. Because sleep problems are becoming increasingly prevalent in the modern society, particularly, when the population is getting older, more profound information on the mechanisms underlying age-related changes is crucial for appropriate treatment.

5. CONCLUSIONS

The main results of the original publications are summarized and shown in figure 5.1.

Figure 5.1. Working model and summary of major findings



Wake and cortical arousal promoting BF activation (SD and NMDA) elevated extracellular adenosine and lactate levels and increased the subsequent sleep. Blocking NMDA receptor activation during SD reduced cortical arousal, as measured by HF EEG theta power, and attenuated subsequent sleep. Induction of iNOS during SD was accompanied with AMPK activation in the BF, but not in the cortex, implicating that the BF is selectively sensitive to the metabolic demands of prolonged waking. In aging, the increases in HF-theta power, and extracellular lactate, adenosine and NO levels were attenuated during SD. NMDA activation did not increase cortical arousal or BF lactate release. Also the sleep responses to both SD and to local BF infusion of NO were attenuated.

These findings implicate that increased or continuous BF activity is important for sustaining cortical arousal during prolonged waking and that BF activation also contributes to the generation of homeostatic sleep pressure. In aging these mechanisms are impaired.

ACKNOWLEDGEMENTS

This work was carried out during the years 2003 to 2008 at the University of Helsinki, Institute of Biomedicine, Department of Physiology. I wish to thank the heads of the Department for providing the research facilities. I would also like to thank the Finnish Graduate School of Neuroscience for financial support and the follow-up team including Esa Korpi and Pertti Panula for overseeing the progress during the final years.

I express my deepest gratitude to everyone around me who encouraged and helped me out during these years. This truly was a team effort. I wish to thank:

First and foremost Tarja Stenberg, my outstanding mentor. I have been extremely fortunate to learn from a true scientist and a teacher. Over the years she maintained a sound balance between pressuring deadlines and support. I was allowed to implement some far fetched ideas as well as to make my own mistakes. I got a comprehensive introduction to science as a profession. For this and everything else I am forever grateful.

Tomi Taira and Leonard Khiroug for kindly reviewing the thesis and for their insightful comments.

The senior scientists and post-doctorate fellows in our team. The late Dag Stenberg, from whom I learned that the best lab instruments are self-made and that with time and devotion, it is possible to know almost everything about sleep reseacrh. Lauri Alanko for teaching me the lab basics and later for stimulating intellectual discussions. Anna Kalinchuk for friendship, guidance, conference company and introduction to the international sleep research community. Andrey Kostin for help in improving our recording facilities and for company in the lab during many long weekends of research. Anna Sofia Urrila for friendship and support.

All the co-authors. In particular Marloes Schepens, Vallo Matto and Kirsi-Marja Rytkönen for their invaluable efforts in data collection and analysis. Without Kirsi-Marja I would still be working with the aging project. Later it was a joy to edit the manuscripts with Kirsi-Marja.

All the past and present members of the Sleep Team Helsinki for making the team feel like a family. In particular I'd like to thank Ernst Mecke for his indispensable help in the lab, for

language revision and for company. Pirjo Saarelainen for patience in carrying out even the oddest ideas when optimizing new methods. Maija Vainikka for sharing enthusiasm for science and for friendsip. I also note Sari Levo-Siitari, Natalia Gass, Janneke Zant, Tiina Paunio, Markus Lagus, Hanna Ollila, Ziddeshwar Utge, Wessel Van Leuwen and Leena Laine and owe my gratitute to several students for their help with sleep scoring.

Marja-Liisa Tiainen and Anja Talvitie for excellent secreterial assistance. Katriina Lauren for help in finding forgotten instruments.

My Ahvis-birdwatching-team, Jaakko and Mirkka Sarparanta and Riksu Hyvönen, for detaching me from the lab every Spring just for the joy of being sleep-deprived and half-frozen in the Åland Islands during the hunt for “lifers”. Janne Turunen for friendship and introduction to academic traditions.

My dear friends and family for always being there and supporting me unconditionally. In particular Maarit, Outi and my three favorite brothers. My parents for unlimited love and support, without you I could not have succeeded.

Finally Kuura and Samu, my companions in life. Kuura for canine companion and for teaching me in practice the importance of routines and rhythms in life. Samu for introduction to script writing, for sharing this experience and for counterbalancing me in so many ways.

Henna-Kaisa Wigren

Helsinki, January 2009

REFERENCES

Abe Y, Toyosawa K (1999) Age-related changes in rat hippocampal theta rhythms: a difference between type 1 and type 2 theta. J Vet Med Sci 61: 543-548.

Achermann P, Borbely AA (2003) Mathematical models of sleep regulation. Front Biosci 8: s683-s693.

Aeschbach D, Matthews JR, Postolache TT, Jackson MA, Giesen HA, Wehr TA (1997) Dynamics of the human EEG during prolonged wakefulness: evidence for frequency-specific circadian and homeostatic influences. Neurosci Lett 239: 121-124.

Aeschbach D, Matthews JR, Postolache TT, Jackson MA, Giesen HA, Wehr TA (1999) Two circadian rhythms in the human electroencephalogram during wakefulness. Am J Physiol 277: R1771-R1779.

Alam MN, Szymusiak R, Gong H, King J, McGinty D (1999) Adenosinergic modulation of rat basal forebrain neurons during sleep and waking: neuronal recording with microdialysis. J Physiol 521 Pt 3: 679-690.

Alanko L, Heiskanen S, Stenberg D, Porkka-Heiskanen T (2003a) Adenosine kinase and 5'-nucleotidase activity after prolonged wakefulness in the cortex and the basal forebrain of rat. Neurochem Int 42: 449-454.

Alanko L, Stenberg D, Porkka-Heiskanen T (2003b) Nitrobenzylthioinosine (NBMPR) binding and nucleoside transporter ENT1 mRNA expression after prolonged wakefulness and recovery sleep in the cortex and basal forebrain of rat. J Sleep Res 12: 299-304.

Alderton WK, Cooper CE, Knowles RG (2001) Nitric oxide synthases: structure, function and inhibition. Biochem J 357: 593-615.

Almeida A, Almeida J, Bolanos JP, Moncada S (2001) Different responses of astrocytes and neurons to nitric oxide: the role of glycolytically generated ATP in astrocyte protection. Proc Natl Acad Sci U S A 98: 15294-15299.

Almeida A, Moncada S, Bolanos JP (2004) Nitric oxide switches on glycolysis through the AMP protein kinase and 6-phosphofructo-2-kinase pathway. Nat Cell Biol 6: 45-51.

Amzica F, Steriade M (1998) Electrophysiological correlates of sleep delta waves. Electroencephalogr Clin Neurophysiol 107: 69-83.

Ancoli-Israel S, Martin JL (2006) Insomnia and daytime napping in older adults. J Clin Sleep Med 2: 333-342.

Apartis E, Poindessous-Jazat F, Epelbaum J, Bassant MH (2000) Age-related changes in rhythmically bursting activity in the medial septum of rats. Brain Res 876: 37-47.

Armstrong DM, Saper CB, Levey AI, Wainer BH, Terry RD (1983) Distribution of cholinergic neurons in rat brain: demonstrated by the immunocytochemical localization of choline acetyltransferase. J Comp Neurol 216: 53-68.

Armstrong DM, Sheffield R, Buzsaki G, Chen KS, Hersh LB, Nearing B, Gage FH (1993) Morphologic alterations of choline acetyltransferase-positive neurons in the basal forebrain of aged behaviorally characterized Fisher 344 rats. Neurobiol Aging 14: 457-470.

Arrigoni E, Chamberlin NL, Saper CB, McCarley RW (2006) Adenosine inhibits basal forebrain cholinergic and noncholinergic neurons in vitro. Neuroscience 140: 403-413.

Attwell D, Gibb A (2005) Neuroenergetics and the kinetic design of excitatory synapses. Nat Rev Neurosci 6: 841-849.

Attwell D, Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab 21: 1133-1145.

Babiloni C, Babiloni F, Carducci F, Cappa SF, Cincotti F, Del Percio C, Miniussi C, Vito MD, Rossi S, Sosta K, Rossini PM (2004) Human cortical rhythms during visual delayed choice reaction time tasks. A high-resolution EEG study on normal aging. Behav Brain Res 153: 261-271.

Basheer R, Arrigoni E, Thatte HS, Greene RW, Ambudkar IS, McCarley RW (2002) Adenosine induces inositol 1,4,5-trisphosphate receptor-mediated mobilization of intracellular calcium stores in basal forebrain cholinergic neurons. J Neurosci 22: 7680-7686.

Basheer R, Rainnie DG, Porkka-Heiskanen T, Ramesh V, McCarley RW (2001) Adenosine, prolonged wakefulness, and A1-activated NF-kappaB DNA binding in the basal forebrain of the rat. Neuroscience 104: 731-739.

Basheer R, Strecker RE, Thakkar MM, McCarley RW (2004) Adenosine and sleep-wake regulation. Prog Neurobiol 73: 379-396.

Baskerville KA, Kent C, Personett D, Lai WR, Park PJ, Coleman P, McKinney M (2008) Aging elevates metabolic gene expression in brain cholinergic neurons. Neurobiol Aging 29: 1874-1893.

Bassant MH, Poindessous-Jazat F (2002) Sleep-related increase in activity of mesopontine neurons in old rats. Neurobiol Aging 23: 615-624.

Beckman KB, Ames BN (1998) The free radical theory of aging matures. Physiol Rev 78: 547-581.

Benington JH, Heller HC (1995) Restoration of brain energy metabolism as the function of sleep. Prog Neurobiol 45: 347-360.

Benington JH, Kodali SK, Heller HC (1995) Stimulation of A1 adenosine receptors mimics the electroencephalographic effects of sleep deprivation. Brain Res 692: 79-85.

Bina KG, Rusak B, Semba K (1993) Localization of cholinergic neurons in the forebrain and brainstem that project to the suprachiasmatic nucleus of the hypothalamus in rat. J Comp Neurol 335: 295-307.

Blanco-Centurion C, Xu M, Murillo-Rodriguez E, Gerashchenko D, Shiromani AM, Salin-Pascual RJ, Hof PR, Shiromani PJ (2006) Adenosine and sleep homeostasis in the Basal forebrain. J Neurosci 26: 8092-8100.

Bland BH (1986) The physiology and pharmacology of hippocampal formation theta rhythms. Prog Neurobiol 26: 1-54.

Bliwise DL (1993) Sleep in normal aging and dementia. Sleep 16: 40-81.

Blois R, Feinberg I, Gaillard JM, Kupfer DJ, Webb WB (1983) Sleep in normal and pathological aging. Experientia 39: 551-558.

Bolanos JP, Cidad P, Garcia-Nogales P, Delgado-Esteban M, Fernandez E, Almeida A (2004) Regulation of glucose metabolism by nitrosative stress in neural cells. Mol Aspects Med 25: 61-73.

Bolanos JP, Delgado-Esteban M, Herrero-Mendez A, Fernandez-Fernandez S, Almeida A (2008) Regulation of glycolysis and pentose-phosphate pathway by nitric oxide: Impact on neuronal survival. Biochim Biophys Acta 1777: 789-793.

Bonnet MH, Arand DL (2007) EEG arousal norms by age. J Clin Sleep Med 3: 271-274.

Borbely AA (1982) A two process model of sleep regulation. Hum Neurobiol 1: 195-204.

Borbely AA, Baumann F, Brandeis D, Strauch I, Lehmann D (1981) Sleep deprivation: effect on sleep stages and EEG power density in man. Electroencephalogr Clin Neurophysiol 51: 483-495.

Borbely AA, Tobler I (1989) Endogenous sleep-promoting substances and sleep regulation. Physiol Rev 69: 605-670.

Born J, Rasch B, Gais S (2006) Sleep to remember. Neuroscientist 12: 410-424.

Borst JG, Leung LW, MacFabe DF (1987) Electrical activity of the cingulate cortex. II. Cholinergic modulation. Brain Res 407: 81-93.

Braun AR, Balkin TJ, Wesenten NJ, Carson RE, Varga M, Baldwin P, Selbie S, Belenky G, Herscovitch P (1997) Regional cerebral blood flow throughout the sleep-wake cycle. An H2(15)O PET study. Brain 120 (Pt 7): 1173-1197.

Brown AM, Ransom BR (2007) Astrocyte glycogen and brain energy metabolism. GLIA 55: 1263-1271.

Brundege JM, Dunwiddie TV (1998) Metabolic regulation of endogenous adenosine release from single neurons. Neuroreport 9: 3007-3011.

Buchsbaum MS, Gillin JC, Wu J, Hazlett E, Sicotte N, Dupont RM, Bunney WE, Jr. (1989) Regional cerebral glucose metabolic rate in human sleep assessed by positron emission tomography. Life Sci 45: 1349-1356.

Burke SN, Barnes CA (2006) Neural plasticity in the ageing brain. Nat Rev Neurosci 7: 30-40.

Burlet S, Cespuglio R (1997) Voltammetric detection of nitric oxide (NO) in the rat brain: its variations throughout the sleep-wake cycle. Neurosci Lett 226: 131-135.

Buzsaki G (2006) Rhythms of the brain. Oxford University Press, Inc., New York

Buzsaki G (2005) Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory. Hippocampus 15: 827-840.

Buzsaki G, Bickford RG, Armstrong DM, Ponomareff G, Chen KS, Ruiz R, Thal LJ, Gage FH (1988a) Electric activity in the neocortex of freely moving young and aged rats. Neuroscience 26: 735-744.

Buzsaki G, Bickford RG, Ponomareff G, Thal LJ, Mandel R, Gage FH (1988b) Nucleus basalis and thalamic control of neocortical activity in the freely moving rat. J Neurosci 8: 4007-4026.

Buzsaki G, Gage FH (1989) The cholinergic nucleus basalis: a key structure in neocortical arousal. EXS 57: 159-171.

Caesar K, Hashemi P, Douhou A, Bonvento G, Boutelle MG, Walls AB, Lauritzen M (2008) Glutamate receptor-dependent increments in lactate, glucose and oxygen metabolism evoked in rat cerebellum in vivo. J Physiol 586: 1337-1349.

Cajochen C, Brunner DP, Krauchi K, Graw P, Wirz-Justice A (1995) Power density in theta/alpha frequencies of the waking EEG progressively increases during sustained wakefulness. Sleep 18: 890-894.

Cajochen C, Foy R, Dijk DJ (1999) Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. Sleep Res Online 2: 65-69.

Cajochen C, Knoblauch V, Krauchi K, Renz C, Wirz-Justice A (2001) Dynamics of frontal EEG activity, sleepiness and body temperature under high and low sleep pressure. Neuroreport 12: 2277-2281.

Cajochen C, Munch M, Knoblauch V, Blatter K, Wirz-Justice A (2006) Age-related changes in the circadian and homeostatic regulation of human sleep. Chronobiol Int 23: 461-474.

Cajochen C, Wyatt JK, Czeisler CA, Dijk DJ (2002) Separation of circadian and wake duration-dependent modulation of EEG activation during wakefulness. Neuroscience 114: 1047-1060.

Calabrese V, Mancuso C, Calvani M, Rizzarelli E, Butterfield DA, Stella AM (2007) Nitric oxide in the central nervous system: neuroprotection versus neurotoxicity. Nat Rev Neurosci 8: 766-775.

Canuelo A, Siles E, Martinez-Romero R, Peinado MA, Martinez-Lara E (2007) The nitric oxide system response to hypoxia/reoxygenation in the aged cerebral cortex. Exp Gerontol 42: 1137-1145.

Cape EG, Jones BE (2000) Effects of glutamate agonist versus procaine microinjections into the basal forebrain cholinergic cell area upon gamma and theta EEG activity and sleep-wake state. Eur J Neurosci 12: 2166-2184.

Carling D (2007) The role of the AMP-activated protein kinase in the regulation of energy homeostasis. Novartis Found Symp 286: 72-81.

Carrier J, Land S, Buysse DJ, Kupfer DJ, Monk TH (2001) The effects of age and gender on sleep EEG power spectral density in the middle years of life (ages 20-60 years old). Psychophysiology 38: 232-242.

Cavas M, Navarro JF (2006) Effects of selective neuronal nitric oxide synthase inhibition on sleep and wakefulness in the rat. Prog Neuropsychopharmacol Biol Psychiatry 30: 56-67.

Chagoya dS, V, Hernandez MR, Suarez J, Vidrio S, Yanez L, Diaz MM (1993) Day-night variations of adenosine and its metabolizing enzymes in the brain cortex of the rat--possible physiological significance for the energetic homeostasis and the sleep-wake cycle. Brain Res 612: 115-121.

Chamberlin NL, Arrigoni E, Chou TC, Scammell TE, Greene RW, Saper CB (2003) Effects of adenosine on gabaergic synaptic inputs to identified ventrolateral preoptic neurons. Neuroscience 119: 913-918.

Chen L, Majde JA, Krueger JM (2003) Spontaneous sleep in mice with targeted disruptions of neuronal or inducible nitric oxide synthase genes. Brain Res 973: 214-222.

Chih CP, Lipton P, Roberts EL, Jr. (2001) Do active cerebral neurons really use lactate rather than glucose? Trends Neurosci 24: 573-578.

Chih CP, Roberts Jr EL (2003) Energy substrates for neurons during neural activity: a critical review of the astrocyte-neuron lactate shuttle hypothesis. J Cereb Blood Flow Metab 23: 1263-1281.

Cirelli C, Huber R, Gopalakrishnan A, Southard TL, Tononi G (2005) Locus ceruleus control of slow-wave homeostasis. J Neurosci 25: 4503-4511.

Clark RS, Kochanek PM, Obrist WD, Wong HR, Billiar TR, Wisniewski SR, Marion DW (1996) Cerebrospinal fluid and plasma nitrite and nitrate concentrations after head injury in humans. Crit Care Med 24: 1243-1251.

Clarke D.D. and Sokoloff L. Circulation and energy metabolism of the brain. Siegel G.J. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 645-680. 1994. New York, Raven Press Ltd.

Coenen AM (1975) Frequency analysis of rat hippocampal electrical activity. Physiol Behav 14: 391-394.

Colas D, Cespuglio R, Sarda N (2005) Sleep wake profile and EEG spectral power in young or old senescence accelerated mice. Neurobiol Aging 26: 265-273.

Colom LV, Christie BR, Bland BH (1988) Cingulate cell discharge patterns related to hippocampal EEG and their modulation by muscarinic and nicotinic agents. Brain Res 460: 329-338.

Cooper CE (2003) Competitive, reversible, physiological? Inhibition of mitochondrial cytochrome oxidase by nitric oxide. IUBMB Life 55: 591-597.

Cotrina ML, Nedergaard M (2002) Astrocytes in the aging brain. J Neurosci Res 67: 1-10.

Cummins TD, Finnigan S (2007) Theta power is reduced in healthy cognitive aging. Int J Psychophysiol 66: 10-17.

Cunha RA (2008) Different cellular sources and different roles of adenosine: A1 receptor-mediated inhibition through astrocytic-driven volume transmission and synapse-restricted A2A receptor-mediated facilitation of plasticity. Neurochem Int 52: 65-72.

Dang-Vu TT, Desseilles M, Laureys S, Degueldre C, Perrin F, Phillips C, Maquet P, Peigneux P (2005) Cerebral correlates of delta waves during non-REM sleep revisited. Neuroimage 28: 14-21.

Dang-Vu TT, Schabus M, Desseilles M, Albouy G, Boly M, Darsaud A, Gais S, Rauchs G, Sterpenich V, Vandewalle G, Carrier J, Moonen G, Balteau E, Degueldre C, Luxen A, Phillips C, Maquet P (2008) Spontaneous neural activity during human slow wave sleep. Proc Natl Acad Sci U S A 105: 15160-15165.

Dare E, Schulte G, Karovic O, Hammarberg C, Fredholm BB (2007) Modulation of glial cell functions by adenosine receptors. Physiol Behav 92: 15-20.

Datta S, Patterson EH, Siwek DF (1997) Endogenous and exogenous nitric oxide in the pedunculopontine tegmentum induces sleep. Synapse 27: 69-78.

Davis KL, Martin E, Turko IV, Murad F (2001) Novel effects of nitric oxide. Annu Rev Pharmacol Toxicol 41: 203-236.

Detari L, Rasmusson DD, Semba K (1997) Phasic relationship between the activity of basal forebrain neurons and cortical EEG in urethane-anesthetized rat. Brain Res 759: 112-121.

Detari L, Rasmusson DD, Semba K (1999) The role of basal forebrain neurons in tonic and phasic activation of the cerebral cortex. Prog Neurobiol 58: 249-277.

Dijk DJ, Beersma DG, Van den Hoofdakker RH (1989) All night spectral analysis of EEG sleep in young adult and middle-aged male subjects. Neurobiol Aging 10: 677-682.

Dijk DJ, Duffy JF, Czeisler CA (2000) Contribution of circadian physiology and sleep homeostasis to age-related changes in human sleep. Chronobiol Int 17: 285-311.

Dijk DJ, von Schantz M (2005) Timing and consolidation of human sleep, wakefulness, and performance by a symphony of oscillators. J Biol Rhythms 20: 279-290.

Drummond SP, Brown GG, Gillin JC, Stricker JL, Wong EC, Buxton RB (2000) Altered brain response to verbal learning following sleep deprivation. Nature 403: 655-657.

Dunwiddie TV, Hoffer BJ (1980) Adenine nucleotides and synaptic transmission in the in vitro rat hippocampus. Br J Pharmacol 69: 59-68.

Dunwiddie TV, Masino SA (2001) The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 24: 31-55.

Dzoljic MR, de Vries R, van Leeuwen R (1996) Sleep and nitric oxide: effects of 7-nitro indazole, inhibitor of brain nitric oxide synthase. Brain Res 718: 145-150.

Ehlers CL, Kupfer DJ (1989) Effects of age on delta and REM sleep parameters. Electroencephalogr Clin Neurophysiol 72: 118-125.

Elmenhorst D, Meyer PT, Winz OH, Matusch A, Ermert J, Coenen HH, Basheer R, Haas HL, Zilles K, Bauer A (2007) Sleep deprivation increases A1 adenosine receptor binding in the human brain: a positron emission tomography study. J Neurosci 27: 2410-2415.

Fadel J, Sarter M, Bruno JP (1999) Age-related attenuation of stimulated cortical acetylcholine release in basal forebrain-lesioned rats. Neuroscience 90: 793-802.

Faraguna U, Vyazovskiy VV, Nelson AB, Tononi G, Cirelli C (2008) A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. J Neurosci 28: 4088-4095.

Feenstra BW, Holsheimer J (1979) Dipole-like neuronal sources of theta rhythm in dorsal hippocampus, dentate gyrus and cingulate cortex of the urethane-anesthetized rat. Electroencephalogr Clin Neurophysiol 47: 532-538.

Feldberg W, Sherwood SL (1954) Infections of drugs into the lateral ventricle of the cat. J Physiol 123: 148-167.

Fields RD, Burnstock G (2006) Purinergic signalling in neuron-glia interactions. Nat Rev Neurosci 7: 423-436.

Finelli LA, Baumann H, Borbely AA, Achermann P (2000) Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. Neuroscience 101: 523-529.

Fournier GN, Materi LM, Semba K, Rasmusson DD (2004) Cortical acetylcholine release and electroencephalogram activation evoked by ionotropic glutamate receptor agonists in the rat basal forebrain. Neuroscience 123: 785-792.

Fox PT, Raichle ME (1986) Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. Proc Natl Acad Sci U S A 83: 1140-1144.

Fox PT, Raichle ME, Mintun MA, Dence C (1988) Nonoxidative glucose consumption during focal physiologic neural activity. Science 241: 462-464.

Franken P (2007) The quality of waking and process S. Sleep 30: 126-127.

Franken P, Dijk DJ, Tobler I, Borbely AA (1991) Sleep deprivation in rats: effects on EEG power spectra, vigilance states, and cortical temperature. Am J Physiol 261: R198-R208.

Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM (2005b) Adenosine and brain function. Int Rev Neurobiol 63: 191-270.

Fredholm BB, Chen JF, Masino SA, Vaugeois JM (2005a) Actions of adenosine at its receptors in the CNS: insights from knockouts and drugs. Annu Rev Pharmacol Toxicol 45: 385-412.

Fuentealba P, Steriade M (2005) The reticular nucleus revisited: intrinsic and network properties of a thalamic pacemaker. Prog Neurobiol 75: 125-141.

Gallopin T, Fort P, Eggermann E, Cauli B, Luppi PH, Rossier J, Audinat E, Muhlethaler M, Serafin M (2000) Identification of sleep-promoting neurons in vitro. Nature 404: 992-995.

Gallopin T, Luppi PH, Cauli B, Urade Y, Rossier J, Hayaishi O, Lambolez B, Fort P (2005) The endogenous somnogen adenosine excites a subset of sleep-promoting neurons via A2A receptors in the ventrolateral preoptic nucleus. Neuroscience 134: 1377-1390.

Garcia-Matas S, Gutierrez-Cuesta J, Coto-Montes A, Rubio-Acero R, Diez-Vives C, Camins A, Pallas M, Sanfeliu C, Cristofol R (2008) Dysfunction of astrocytes in senescence-accelerated mice SAMP8 reduces their neuroprotective capacity. Aging Cell 7: 630-640.

Garthwaite J (2008) Concepts of neural nitric oxide-mediated transmission. Eur J Neurosci 27: 2783-2802.

Gaudreau H, Morettini J, Lavoie HB, Carrier J (2001) Effects of a 25-h sleep deprivation on daytime sleep in the middle-aged. Neurobiol Aging 22: 461-468.

Gaus SE, Strecker RE, Tate BA, Parker RA, Saper CB (2002) Ventrolateral preoptic nucleus contains sleep-active, galaninergic neurons in multiple mammalian species. Neuroscience 115: 285-294.

Gautier-Sauvigne S, Colas D, Parmantier P, Clement P, Gharib A, Sarda N, Cespuglio R (2005) Nitric oxide and sleep. Sleep Med Rev 9: 101-113.

Giovannini MG, Bartolini L, Kopf SR, Pepeu G (1998) Acetylcholine release from the frontal cortex during exploratory activity. Brain Res 784: 218-227.

Gladden LB (2004) Lactate metabolism: a new paradigm for the third millennium. J Physiol 558: 5-30.

Golmayo L, Nunez A, Zaborszky L (2003) Electrophysiological evidence for the existence of a posterior cortical-prefrontal-basal forebrain circuitry in modulating sensory responses in visual and somatosensory rat cortical areas. Neuroscience 119: 597-609.

Gonzales RA, Brown LM, Jones TW, Trent RD, Westbrook SL, Leslie SW (1991) N-methyl-D-aspartate mediated responses decrease with age in Fischer 344 rat brain. Neurobiol Aging 12: 219-225.

Grewer C, Rauen T (2005) Electrogenic glutamate transporters in the CNS: molecular mechanism, pre-steady-state kinetics, and their impact on synaptic signaling. J Membr Biol 203: 1-20.

Grill JD, Riddle DR (2002) Age-related and laminar-specific dendritic changes in the medial frontal cortex of the rat. Brain Res 937: 8-21.

Gritti I, Henny P, Galloni F, Mainville L, Mariotti M, Jones BE (2006) Stereological estimates of the basal forebrain cell population in the rat, including neurons containing choline acetyltransferase, glutamic acid decarboxylase or phosphate-activated glutaminase and colocalizing vesicular glutamate transporters. Neuroscience 143: 1051-1064.

Gritti I, Mainville L, Mancia M, Jones BE (1997) GABAergic and other noncholinergic basal forebrain neurons, together with cholinergic neurons, project to the mesocortex and isocortex in the rat. J Comp Neurol 383: 163-177.

Grob RL (1985) Modern Practice of Gas Chromatography. New York: John Wiley&Sons.

Guix FX, Uribesalgo I, Coma M, Munoz FJ (2005) The physiology and pathophysiology of nitric oxide in the brain. Prog Neurobiol 76: 126-152.

Haas HL, Selbach O (2000) Functions of neuronal adenosine receptors. Naunyn Schmiedebergs Arch Pharmacol 362: 375-381.

Haberg A, Qu H, Haraldseth O, Unsgard G, Sonnewald U (2000) In vivo effects of adenosine A1 receptor agonist and antagonist on neuronal and astrocytic intermediary metabolism studied with ex vivo 13C NMR spectroscopy. J Neurochem 74: 327-333.

Hallstrom A, Carlsson A, Hillered L, Ungerstedt U (1989) Simultaneous determination of lactate, pyruvate, and ascorbate in microdialysis samples from rat brain, blood, fat, and muscle using high-performance liquid chromatography. J Pharmacol Methods 22: 113-124.

Hardie DG, Hawley SA, Scott JW (2006) AMP-activated protein kinase--development of the energy sensor concept. J Physiol 574: 7-15.

Harman D (1956) Aging: a theory based on free radical and radiation chemistry. J Gerontol 11: 298-300.

Harman D (2006) Free radical theory of aging: an update: increasing the functional life span. Ann N Y Acad Sci 1067: 10-21.

Hars B (1999) Endogenous nitric oxide in the rat pons promotes sleep. Brain Res 816: 209-219.

Hasselmo ME (2006) The role of acetylcholine in learning and memory. Curr Opin Neurobiol 16: 710-715.

Hasselmo ME, Giocomo LM (2006) Cholinergic modulation of cortical function. J Mol Neurosci 30: 133-135.

Haulica I, Ababei L, Branisteanu D, Topoliceanu F (1973) Letter: Preliminary data on the possible hypnogenic role of adenosine. J Neurochem 21: 1019-1020.

Henny P, Jones BE (2008) Projections from basal forebrain to prefrontal cortex comprise cholinergic, GABAergic and glutamatergic inputs to pyramidal cells or interneurons. Eur J Neurosci 27: 654-670.

Hertz L (2004) The astrocyte-neuron lactate shuttle: a challenge of a challenge. J Cereb Blood Flow Metab 24: 1241-1248.

Herzog CD, Nowak KA, Sarter M, Bruno JP (2003) Microdialysis without acetylcholinesterase inhibition reveals an age-related attenuation in stimulated cortical acetylcholine release. Neurobiol Aging 24: 861-863.

Hofle N, Paus T, Reutens D, Fiset P, Gotman J, Evans AC, Jones BE (1997) Regional cerebral blood flow changes as a function of delta and spindle activity during slow wave sleep in humans. J Neurosci 17: 4800-4808.

Hong ZY, Huang ZL, Qu WM, Eguchi N, Urade Y, Hayaishi O (2005) An adenosine A receptor agonist induces sleep by increasing GABA release in the tuberomammillary nucleus to inhibit histaminergic systems in rats. J Neurochem 92: 1542-1549.

Hoyer S (1985) The effect of age on glucose and energy metabolism in brain cortex of rats. Arch Gerontol Geriatr 4: 193-203.

Hoyer S, Lannert H, Latteier E, Meisel T (2004) Relationship between cerebral energy metabolism in parietotemporal cortex and hippocampus and mental activity during aging in rats. J Neural Transm 111: 575-589.

Hu Y, Wilson GS (1997) A temporary local energy pool coupled to neuronal activity: fluctuations of extracellular lactate levels in rat brain monitored with rapid-response enzyme-based sensor. J Neurochem 69: 1484-1490.

Huang ZL, Qu WM, Eguchi N, Chen JF, Schwarzschild MA, Fredholm BB, Urade Y, Hayaishi O (2005) Adenosine A2A, but not A1, receptors mediate the arousal effect of caffeine. Nat Neurosci 8: 858-859.

Huang ZL, Urade Y, Hayaishi O (2007) Prostaglandins and adenosine in the regulation of sleep and wakefulness. Curr Opin Pharmacol 7: 33-38.

Huber R, Tononi G, Cirelli C (2007) Exploratory behavior, cortical BDNF expression, and sleep homeostasis. Sleep 30: 129-139.

Hue L, Rider MH (2007) The AMP-activated protein kinase: more than an energy sensor. Essays Biochem 43: 121-137.

Huston JP, Haas HL, Boix F, Pfister M, Decking U, Schrader J, Schwarting RK (1996) Extracellular adenosine levels in neostriatum and hippocampus during rest and activity periods of rats. Neuroscience 73: 99-107.

IFSECN (1974) A glossary of terms most commonly used by clinical electroencephalographers. Electroencephalogr Clin Neurophysiol 37: 538-548.

Isomura Y, Sirota A, Ozen S, Montgomery S, Mizuseki K, Henze DA, Buzsaki G (2006) Integration and segregation of activity in entorhinal-hippocampal subregions by neocortical slow oscillations. Neuron 52: 871-882.

Ji D, Wilson MA (2007) Coordinated memory replay in the visual cortex and hippocampus during sleep. Nat Neurosci 10: 100-107.

Jones BE (2003) Arousal systems. Front Biosci 8: s438-s451.

Jones BE (2004) Activity, modulation and role of basal forebrain cholinergic neurons innervating the cerebral cortex. Prog Brain Res 145: 157-169.

Jones BE (2008) Modulation of cortical activation and behavioral arousal by cholinergic and orexinergic systems. Ann N Y Acad Sci 1129: 26-34.

Joo HJ, Ma JY, Choo YG, Choi BK, Jung KY (1999) Age-related alteration of intracellular ATP maintenance in the cell suspensions of mice cerebral cortex. Mech Ageing Dev 110: 1-12.

Jung R, Kornmuller A (1939) Eine Methodik der Ableitung lokalisierter Potentialschwankungen aus subcorticalen Hirngebieten. Arch Psychiat Nerv Krank 109: 1-17.

Kahana MJ, Seelig D, Madsen JR (2001) Theta returns. Curr Opin Neurobiol 11: 739-744.

Kahn BB, Alquier T, Carling D, Hardie DG (2005) AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab 1: 15-25.

Kalinchuk AV, Lu Y, Stenberg D, Rosenberg PA, Porkka-Heiskanen T (2006a) Nitric oxide production in the basal forebrain is required for recovery sleep. J Neurochem 99: 483-498.

Kalinchuk AV, McCarley RW, Stenberg D, Porkka-Heiskanen T, Basheer R (2008) The role of cholinergic basal forebrain neurons in adenosine-mediated homeostatic control of sleep: Lessons from 192 IGG-saporin lesions. Neuroscience.

Kalinchuk AV, Stenberg D, Rosenberg PA, Porkka-Heiskanen T (2006b) Inducible and neuronal nitric oxide synthases (NOS) have complementary roles in recovery sleep induction. Eur J Neurosci 24: 1443-1456.

Kalinchuk AV, Urrila AS, Alanko L, Heiskanen S, Wigren HK, Suomela M, Stenberg D, Porkka-Heiskanen T (2003) Local energy depletion in the basal forebrain increases sleep. Eur J Neurosci 17: 863-869.

Kapas L, Fang J, Krueger JM (1994) Inhibition of nitric oxide synthesis inhibits rat sleep. Brain Res 664: 189-196.

Kapas L, Krueger JM (1996) Nitric oxide donors SIN-1 and SNAP promote nonrapid-eye-movement sleep in rats. Brain Res Bull 41: 293-298.

Karnovsky ML, Reich P, Anchors JM, Burrows BL (1983) Changes in brain glycogen during slow-wave sleep in the rat. J Neurochem 41: 1498-1501.

Kasischke KA (2008) A new pathway for lactate production in the CNS. J Physiol 586: 1207-1208.

Kasischke KA, Vishwasrao HD, Fisher PJ, Zipfel WR, Webb WW (2004) Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. Science 305: 99-103.

Kaur S, Junek A, Black MA, Semba K (2008) Effects of ibotenate and 192IgG-saporin lesions of the nucleus basalis magnocellularis/substantia innominata on spontaneous sleep and wake states and on recovery sleep after sleep deprivation in rats. J Neurosci 28: 491-504.

Kennedy C, Gillin JC, Mendelson W, Suda S, Miyaoka M, Ito M, Nakamura RK, Storch FI, Pettigrew K, Mishkin M, Sokoloff L (1982) Local cerebral glucose utilization in non-rapid eye movement sleep. Nature 297: 325-327.

Khateb A, Fort P, Serafin M, Jones BE, Muhlethaler M (1995) Rhythmical bursts induced by NMDA in guinea-pig cholinergic nucleus basalis neurones in vitro. J Physiol 487 (Pt 3): 623-638.

Khateb A, Muhlethaler M, Alonso A, Serafin M, Mainville L, Jones BE (1992) Cholinergic nucleus basalis neurons display the capacity for rhythmic bursting activity mediated by low-threshold calcium spikes. Neuroscience 51: 489-494.

Kleiner S, Bringmann A (1996) Nucleus basalis magnocellularis and pedunculopontine tegmental nucleus: control of the slow EEG waves in rats. Arch Ital Biol 134: 153-167.

Klerman EB, Dijk DJ (2008) Age-related reduction in the maximal capacity for sleep--implications for insomnia. Curr Biol 18: 1118-1123.

Kocsis JD, Eng DL, Bhisitkul RB (1984) Adenosine selectively blocks parallel-fiber-mediated synaptic potentials in rat cerebellar cortex. Proc Natl Acad Sci U S A 81: 6531-6534.

Korf J (2006) Is brain lactate metabolized immediately after neuronal activity through the oxidative pathway? J Cereb Blood Flow Metab 26: 1584-1586.

Kostin A, Stenberg D, Kalinchuk AV, Porkka-Heiskanen T (2008) Nitric oxide modulates the discharge rate of basal forebrain neurons. Psychopharmacology (Berl) 201: 147-160.

Kramis R, Vanderwolf CH, Bland BH (1975) Two types of hippocampal rhythmical slow activity in both the rabbit and the rat: relations to behavior and effects of atropine, diethyl ether, urethane, and pentobarbital. Exp Neurol 49: 58-85.

Kuhr WG, Korf J (1988) Extracellular lactic acid as an indicator of brain metabolism: continuous on-line measurement in conscious, freely moving rats with intrastriatal dialysis. J Cereb Blood Flow Metab 8: 130-137.

Kuramoto N, Wilkins ME, Fairfax BP, Revilla-Sanchez R, Terunuma M, Tamaki K, Iemata M, Warren N, Couve A, Calver A, Horvath Z, Freeman K, Carling D, Huang L, Gonzales C, Cooper E, Smart TG, Pangalos MN, Moss SJ (2007) Phospho-dependent functional modulation of GABA(B) receptors by the metabolic sensor AMP-dependent protein kinase. Neuron 53: 233-247.

Lafrance C, Dumont M (2000) Diurnal variations in the waking EEG: comparisons with sleep latencies and subjective alertness. J Sleep Res 9: 243-248.

Lamour Y, Dutar P, Rascol O, Jobert A (1986) Basal forebrain neurons projecting to the rat frontoparietal cortex: electrophysiological and pharmacological properties. Brain Res 362: 122-131.

Landolt HP (2008) Sleep homeostasis: a role for adenosine in humans? Biochem Pharmacol 75: 2070-2079.

Landolt HP, Borbely AA (2001) Age-dependent changes in sleep EEG topography. Clin Neurophysiol 112: 369-377.

Landolt HP, Dijk DJ, Achermann P, Borbely AA (1996) Effect of age on the sleep EEG: slow-wave activity and spindle frequency activity in young and middle-aged men. Brain Res 738: 205-212.

Landolt HP, Dijk DJ, Gaus SE, Borbely AA (1995) Caffeine reduces low-frequency delta activity in the human sleep EEG. Neuropsychopharmacology 12: 229-238.

Landolt HP, Retey JV, Tonz K, Gottselig JM, Khatami R, Buckelmuller I, Achermann P (2004) Caffeine attenuates waking and sleep electroencephalographic markers of sleep homeostasis in humans. Neuropsychopharmacology 29: 1933-1939.

Latini S, Pedata F (2001) Adenosine in the central nervous system: release mechanisms and extracellular concentrations. J Neurochem 79: 463-484.

Ledo A, Barbosa RM, Gerhardt GA, Cadenas E, Laranjinha J (2005) Concentration dynamics of nitric oxide in rat hippocampal subregions evoked by stimulation of the NMDA glutamate receptor. Proc Natl Acad Sci U S A 102: 17483-17488.

Lee MG, Hassani OK, Alonso A, Jones BE (2005) Cholinergic basal forebrain neurons burst with theta during waking and paradoxical sleep. J Neurosci 25: 4365-4369.

Leenders KL, Perani D, Lammertsma AA, Heather JD, Buckingham P, Healy MJ, Gibbs JM, Wise RJ, Hatazawa J, Herold S, . (1990) Cerebral blood flow, blood volume and oxygen utilization. Normal values and effect of age. Brain 113 (Pt 1): 27-47.

Leonard TO, Lydic R (1995) Nitric oxide synthase inhibition decreases pontine acetylcholine release. Neuroreport 6: 1525-1529.

Lloyd HG, Lindstrom K, Fredholm BB (1993) Intracellular formation and release of adenosine from rat hippocampal slices evoked by electrical stimulation or energy depletion. Neurochem Int 23: 173-185.

Lorenzo I, Ramos J, Arce C, Guevara MA, Corsi-Cabrera M (1995) Effect of total sleep deprivation on reaction time and waking EEG activity in man. Sleep 18: 346-354.

Lu J, Bjorkum AA, Xu M, Gaus SE, Shiromani PJ, Saper CB (2002) Selective activation of the extended ventrolateral preoptic nucleus during rapid eye movement sleep. J Neurosci 22: 4568-4576.

Lu J, Greco MA, Shiromani P, Saper CB (2000) Effect of lesions of the ventrolateral preoptic nucleus on NREM and REM sleep. J Neurosci 20: 3830-3842.

Luiten PG, Gaykema RP, Traber J, Spencer DG, Jr. (1987) Cortical projection patterns of magnocellular basal nucleus subdivisions as revealed by anterogradely transported Phaseolus vulgaris leucoagglutinin. Brain Res 413: 229-250.

Mackenzie IM, Ekangaki A, Young JD, Garrard CS (1996) Effect of renal function on serum nitrogen oxide concentrations. Clin Chem 42: 440-444.

Mackiewicz M, Nikonova EV, Zimmerman JE, Galante RJ, Zhang L, Cater JR, Geiger JD, Pack AI (2003) Enzymes of adenosine metabolism in the brain: diurnal rhythm and the effect of sleep deprivation. J Neurochem 85: 348-357.

Madrigal JL, Moro MA, Lizasoain I, Lorenzo P, Castrillo A, Bosca L, Leza JC (2001) Inducible nitric oxide synthase expression in brain cortex after acute restraint stress is regulated by nuclear factor kappaB-mediated mechanisms. J Neurochem 76: 532-538.

Madsen PL, Schmidt JF, Wildschiodtz G, Friberg L, Holm S, Vorstrup S, Lassen NA (1991) Cerebral O2 metabolism and cerebral blood flow in humans during deep and rapid-eye-movement sleep. J Appl Physiol 70: 2597-2601.

Manfridi A, Brambilla D, Mancia M (1999) Stimulation of NMDA and AMPA receptors in the rat nucleus basalis of Meynert affects sleep. Am J Physiol 277: R1488-R1492.

Mangia S, Garreffa G, Bianciardi M, Giove F, Di Salle F, Maraviglia B (2003) The aerobic brain: lactate decrease at the onset of neural activity. Neuroscience 118: 7-10.

Manns ID, Alonso A, Jones BE (2000) Discharge profiles of juxtacellularly labeled and immunohistochemically identified GABAergic basal forebrain neurons recorded in association with the electroencephalogram in anesthetized rats. J Neurosci 20: 9252-9263.

Maquet P, Dive D, Salmon E, Sadzot B, Franco G, Poirrier R, von Frenckell R, Franck G (1990) Cerebral glucose utilization during sleep-wake cycle in man determined by positron emission tomography and [18F]2-fluoro-2-deoxy-D-glucose method. Brain Res 513: 136-143.

Marshall L, Helgadottir H, Molle M, Born J (2006) Boosting slow oscillations during sleep potentiates memory. Nature 444: 610-613.

Martin AJ, Friston KJ, Colebatch JG, Frackowiak RS (1991) Decreases in regional cerebral blood flow with normal aging. J Cereb Blood Flow Metab 11: 684-689.

Massieu L, Morales-Villagran A, Tapia R (1995) Accumulation of extracellular glutamate by inhibition of its uptake is not sufficient for inducing neuronal damage: an in vivo microdialysis study. J Neurochem 64: 2262-2272.

Massimini M, Ferrarelli F, Esser SK, Riedner BA, Huber R, Murphy M, Peterson MJ, Tononi G (2007) Triggering sleep slow waves by transcranial magnetic stimulation. Proc Natl Acad Sci U S A 104: 8496-8501.

McCann SM, Mastronardi C, de Laurentiis A, Rettori V (2005) The nitric oxide theory of aging revisited. Ann N Y Acad Sci 1057: 64-84.

McCarley RW (2007) Neurobiology of REM and NREM sleep. Sleep Med 8: 302-330.

McEvoy LK, Pellouchoud E, Smith ME, Gevins A (2001) Neurophysiological signals of working memory in normal aging. Brain Res Cogn Brain Res 11: 363-376.

McEwen BS (2000) Allostasis, allostatic load, and the aging nervous system: role of excitatory amino acids and excitotoxicity. Neurochem Res 25: 1219-1231.

McGinty D, Gong H, Suntsova N, Alam MN, Methippara M, Guzman-Marin R, Szymusiak R (2004) Sleep-promoting functions of the hypothalamic median preoptic nucleus: inhibition of arousal systems. Arch Ital Biol 142: 501-509.

McKinney M, Jacksonville MC (2005) Brain cholinergic vulnerability: relevance to behavior and disease. Biochem Pharmacol 70: 1115-1124.

McKinney M, Williams K, Personett D, Kent C, Bryan D, Gonzalez J, Baskerville K (2004) Pontine cholinergic neurons depend on three neuroprotection systems to resist nitrosative stress. Brain Res 1002: 100-109.

McNaughton N, Sedgwick EM (1978) Reticular stimulation and hippocampal theta rhythm in rats: effects of drugs. Neuroscience 3: 629-632.

Melov S (2004) Modeling mitochondrial function in aging neurons. Trends Neurosci 27: 601-606.

Mendelson WB, Bergmann BM (1999a) Age-related changes in sleep in the rat. Sleep 22: 145-150.

Mendelson WB, Bergmann BM (1999b) EEG delta power during sleep in young and old rats. Neurobiol Aging 20: 669-673.

Mendelson WB, Bergmann BM (2000) Age-dependent changes in recovery sleep after 48 hours of sleep deprivation in rats. Neurobiol Aging 21: 689-693.

Mesulam MM, Mufson EJ, Wainer BH, Levey AI (1983) Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience 10: 1185-1201.

Methippara MM, Kumar S, Alam MN, Szymusiak R, McGinty D (2005) Effects on sleep of microdialysis of adenosine A1 and A2a receptor analogs into the lateral preoptic area of rats. Am J Physiol Regul Integr Comp Physiol 289: R1715-R1723.

Miles LE, Dement WC (1980) Sleep and aging. Sleep 3: 1-220.

Miquel J (1998) An update on the oxygen stress-mitochondrial mutation theory of aging: genetic and evolutionary implications. Exp Gerontol 33: 113-126.

Mitchell DJ, McNaughton N, Flanagan D, Kirk IJ (2008) A review of frontal-midline theta from the perspective of hippocampal "THETA". Prog Neurobiol.

Mitsushima D, Mizuno T, Kimura F (1996) Age-related changes in diurnal acetylcholine release in the prefrontal cortex of male rats as measured by microdialysis. Neuroscience 72: 429-434.

Modirrousta M, Mainville L, Jones BE (2004) Gabaergic neurons with alpha2-adrenergic receptors in basal forebrain and preoptic area express c-Fos during sleep. Neuroscience 129: 803-810.

Moeller JR, Ishikawa T, Dhawan V, Spetsieris P, Mandel F, Alexander GE, Grady C, Pietrini P, Eidelberg D (1996) The metabolic topography of normal aging. J Cereb Blood Flow Metab 16: 385-398.

Molle M, Yeshenko O, Marshall L, Sara SJ, Born J (2006) Hippocampal sharp wave-ripples linked to slow oscillations in rat slow-wave sleep. J Neurophysiol 96: 62-70.

Moncada S, Bolanos JP (2006) Nitric oxide, cell bioenergetics and neurodegeneration. J Neurochem 97: 1676-1689.

Monti JM, Hantos H, Ponzoni A, Monti D, Banchero P (1999) Role of nitric oxide in sleep regulation: effects of L-NAME, an inhibitor of nitric oxide synthase, on sleep in rats. Behav Brain Res 100: 197-205.

Monti JM, Jantos H (2004a) Effects of L-arginine and SIN-1 on sleep and waking in the rat during both phases of the light-dark cycle. Life Sci 75: 2027-2034.

Monti JM, Jantos H (2004b) Microinjection of the nitric oxide synthase inhibitor L-NAME into the lateral basal forebrain alters the sleep/wake cycle of the rat. Prog Neuropsychopharmacol Biol Psychiatry 28: 239-247.

Monti JM, Jantos H, Monti D (2001) Increase of waking and reduction of NREM and REM sleep after nitric oxide synthase inhibition: prevention with GABA(A) or adenosine A(1) receptor agonists. Behav Brain Res 123: 23-35.

Moore RY (2007) Suprachiasmatic nucleus in sleep-wake regulation. Sleep Med 8 Suppl 3: 27-33.

Morairty S, Rainnie D, McCarley R, Greene R (2004) Disinhibition of ventrolateral preoptic area sleep-active neurons by adenosine: a new mechanism for sleep promotion. Neuroscience 123: 451-457.

Moruzzi G, Magoun HW (1949) Brain stem reticular formation and activation of the EEG. Electroencephalogr Clin Neurophysiol 1: 455-473.

Mourtazaev MS, Kemp B, Zwinderman AH, Kamphuisen HA (1995) Age and gender affect different characteristics of slow waves in the sleep EEG. Sleep 18: 557-564.

Munch M, Knoblauch V, Blatter K, Schroder C, Schnitzler C, Krauchi K, Wirz-Justice A, Cajochen C (2004) The frontal predominance in human EEG delta activity after sleep loss decreases with age. Eur J Neurosci 20: 1402-1410.

Murchison D, Griffith WH (2007) Calcium buffering systems and calcium signaling in aged rat basal forebrain neurons. Aging Cell 6: 297-305.

Murillo-Rodriguez E, Blanco-Centurion C, Gerashchenko D, Salin-Pascual RJ, Shiromani PJ (2004) The diurnal rhythm of adenosine levels in the basal forebrain of young and old rats. Neuroscience 123: 361-370.

Murphy S (2000) Production of nitric oxide by glial cells: regulation and potential roles in the CNS. GLIA 29: 1-13.

Nakamura T, Gu Z, Lipton SA (2007) Contribution of glutamatergic signaling to nitrosative stress-induced protein misfolding in normal brain aging and neurodegenerative diseases. Aging Cell 6: 351-359.

Navarro A, Boveris A (2007) The mitochondrial energy transduction system and the aging process. Am J Physiol Cell Physiol 292: C670-C686.

Navarro A, Lopez-Cepero JM, Bandez MJ, Sanchez-Pino MJ, Gomez C, Cadenas E, Boveris A (2008) Hippocampal mitochondrial dysfunction in rat aging. Am J Physiol Regul Integr Comp Physiol 294: R501-R509.

Netchiporouk L, Shram N, Salvert D, Cespuglio R (2001) Brain extracellular glucose assessed by voltammetry throughout the rat sleep-wake cycle. Eur J Neurosci 13: 1429-1434.

Nikonova EV, Vijayasarathy C, Zhang L, Cater JR, Galante RJ, Ward SE, Avadhani NG, Pack AI (2005) Differences in activity of cytochrome C oxidase in brain between sleep and wakefulness. Sleep 28: 21-27.

Nunez, P. L. and Srinivasan, R. Electric Fields of the Brain. The Neurophysics of EEG. Second Edition. 2006. New York, Oxford University Press.

Obal F, Jr., Krueger JM (2003) Biochemical regulation of non-rapid-eye-movement sleep. Front Biosci 8: d520-d550.

Ohayon MM, Carskadon MA, Guilleminault C, Vitiello MV (2004) Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. Sleep 27: 1255-1273.

Olejniczak P (2006) Neurophysiologic basis of EEG. J Clin Neurophysiol 23: 186-189.

Opp MR, Krueger JM (1994) Anti-interleukin-1 beta reduces sleep and sleep rebound after sleep deprivation in rats. Am J Physiol 266: R688-R695.

Palacios-Callender M, Hollis V, Frakich N, Mateo J, Moncada S (2007) Cytochrome c oxidase maintains mitochondrial respiration during partial inhibition by nitric oxide. J Cell Sci 120: 160-165.

Palomba M, Nygard M, Florenzano F, Bertini G, Kristensson K, Bentivoglio M (2008) Decline of the presynaptic network, including GABAergic terminals, in the aging suprachiasmatic nucleus of the mouse. J Biol Rhythms 23: 220-231.

Parikh V, Kozak R, Martinez V, Sarter M (2007) Prefrontal acetylcholine release controls cue detection on multiple timescales. Neuron 56: 141-154.

Parikh V, Sarter M (2008) Cholinergic mediation of attention: contributions of phasic and tonic increases in prefrontal cholinergic activity. Ann N Y Acad Sci 1129: 225-235.

Pawlyk AC, Ferber M, Shah A, Pack AI, Naidoo N (2007) Proteomic analysis of the effects and interactions of sleep deprivation and aging in mouse cerebral cortex. J Neurochem 103: 2301-2313.

Paxinos G, Watson C (1998) The Rat Brain in Stereotaxic Coordinates. San Diego: Academic Press Inc.

Pellerin L, Bouzier-Sore AK, Aubert A, Serres S, Merle M, Costalat R, Magistretti PJ (2007) Activity-dependent regulation of energy metabolism by astrocytes: an update. GLIA 55: 1251-1262.

Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. PNAS 91: 10625-10629.

Pellerin L, Magistretti PJ (2004) Neuroscience. Let there be (NADH) light. Science 305: 50-52.

Personett D, Fass U, Panickar K, McKinney M (2000) Retinoic acid-mediated enhancement of the cholinergic/neuronal nitric oxide synthase phenotype of the medial septal SN56 clone: establishment of a nitric oxide-sensitive proapoptotic state. J Neurochem 74: 2412-2424.

Petit-Taboue MC, Landeau B, Desson JF, Desgranges B, Baron JC (1998) Effects of healthy aging on the regional cerebral metabolic rate of glucose assessed with statistical parametric mapping. Neuroimage 7: 176-184.

Pinto-Duarte A, Coelho JE, Cunha RA, Ribeiro JA, Sebastiao AM (2005) Adenosine A2A receptors control the extracellular levels of adenosine through modulation of nucleoside transporters activity in the rat hippocampus. J Neurochem 93: 595-604.

Poon HF, Vaishnav RA, Getchell TV, Getchell ML, Butterfield DA (2006) Quantitative proteomics analysis of differential protein expression and oxidative modification of specific proteins in the brains of old mice. Neurobiol Aging 27: 1010-1019.

Porkka-Heiskanen T, Alanko L, Kalinchuk A, Stenberg D (2002) Adenosine and sleep. Sleep Med Rev 6: 321-332.

Porkka-Heiskanen T, Strecker RE, McCarley RW (2000) Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep: an in vivo microdialysis study. Neuroscience 99: 507-517.

Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjorkum AA, Greene RW, McCarley RW (1997) Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. Science 276: 1265-1268.

Portas CM, Thakkar M, Rainnie DG, Greene RW, McCarley RW (1997) Role of adenosine in behavioral state modulation: a microdialysis study in the freely moving cat. Neuroscience 79: 225-235.

Prichard J, Rothman D, Novotny E, Petroff O, Kuwabara T, Avison M, Howseman A, Hanstock C, Shulman R (1991) Lactate rise detected by 1H NMR in human visual cortex during physiologic stimulation. Proc Natl Acad Sci U S A 88: 5829-5831.

Radulovacki M (1985) Role of adenosine in sleep in rats. Rev Clin Basic Pharm 5: 327-339.

Raichle ME, Mintun MA (2006) Brain work and brain imaging. Annu Rev Neurosci 29: 449-476.

Raichle ME, Posner JB, Plum F (1970) Cerebral blood flow during and after hyperventilation. Arch Neurol 23: 394-403.

Ramesh V, Thatte HS, McCarley RW, Basheer R (2007) Adenosine and sleep deprivation promote NF-kappaB nuclear translocation in cholinergic basal forebrain. J Neurochem 100: 1351-1363.

Ramm P, Frost BJ (1986) Cerebral and local cerebral metabolism in the cat during slow wave and REM sleep. Brain Res 365: 112-124.

Raz N, Gunning FM, Head D, Dupuis JH, McQuain J, Briggs SD, Loken WJ, Thornton AE, Acker JD (1997) Selective aging of the human cerebral cortex observed in vivo: differential vulnerability of the prefrontal gray matter. Cereb Cortex 7: 268-282.

Rebola N, Rodrigues RJ, Lopes LV, Richardson PJ, Oliveira CR, Cunha RA (2005) Adenosine A1 and A2A receptors are co-expressed in pyramidal neurons and co-localized in glutamatergic nerve terminals of the rat hippocampus. Neuroscience 133: 79-83.

Reich P, Geyer SJ, Karnovsky ML (1972) Metabolism of brain during sleep and wakefulness. J Neurochem 19: 487-497.

Retey JV, Adam M, Honegger E, Khatami R, Luhmann UF, Jung HH, Berger W, Landolt HP (2005) A functional genetic variation of adenosine deaminase affects the duration and intensity of deep sleep in humans. Proc Natl Acad Sci U S A 102: 15676-15681.

Retey JV, Adam M, Khatami R, Luhmann UF, Jung HH, Berger W, Landolt HP (2007) A genetic variation in the adenosine A2A receptor gene (ADORA2A) contributes to individual sensitivity to caffeine effects on sleep. Clin Pharmacol Ther 81: 692-698.

Ribeiro AC, Gilligan JG, Kapas L (2000) Systemic injection of a nitric oxide synthase inhibitor suppresses sleep responses to sleep deprivation in rats. Am J Physiol Regul Integr Comp Physiol 278: R1048-R1056.

Ribeiro AC, Kapas L (2005) Day- and nighttime injection of a nitric oxide synthase inhibitor elicits opposite sleep responses in rats. Am J Physiol Regul Integr Comp Physiol 289: R521-R531.

Richter D, Dawson RM (1948) Brain metabolism in emotional excitement and in sleep. Am J Physiol 154: 73-79.

Ros J, Pellerin L, Magara F, Dauguet J, Schenk F, Magistretti PJ (2006) Metabolic activation pattern of distinct hippocampal subregions during spatial learning and memory retrieval. J Cereb Blood Flow Metab 26: 468-477.

Rosenberg PA, Li Y, Le M, Zhang Y (2000) Nitric oxide-stimulated increase in extracellular adenosine accumulation in rat forebrain neurons in culture is associated with ATP hydrolysis and inhibition of adenosine kinase activity. J Neurosci 20: 6294-6301.

Rosenberg RS, Bergmann BM, Rechtschaffen A (1976) Variations in slow wave activity during sleep in the rat. Physiol Behav 17: 931-938.

Rosenberg RS, Zepelin H, Rechtschaffen A (1979) Sleep in young and old rats. J Gerontol 34: 525-532.

Rossini PM, Rossi S, Babiloni C, Polich J (2007) Clinical neurophysiology of aging brain: from normal aging to neurodegeneration. Prog Neurobiol 83: 375-400.

Roy CS, Sherrington CS (1890) On the Regulation of the Blood-supply of the Brain. J Physiol 11: 85-158.

Sagar SM, Sharp FR, Curran T (1988) Expression of c-fos protein in brain: metabolic mapping at the cellular level. Science 240: 1328-1331.

Saper CB, Scammell TE, Lu J (2005) Hypothalamic regulation of sleep and circadian rhythms. Nature 437: 1257-1263.

Sappey-Marinier D, Calabrese G, Fein G, Hugg JW, Biggins C, Weiner MW (1992) Effect of photic stimulation on human visual cortex lactate and phosphates using 1H and 31P magnetic resonance spectroscopy. J Cereb Blood Flow Metab 12: 584-592.

Sarter M, Bruno JP (1997) Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. Brain Res Brain Res Rev 23: 28-46.

Sarter M, Bruno JP (2004) Developmental origins of the age-related decline in cortical cholinergic function and associated cognitive abilities. Neurobiol Aging 25: 1127-1139.

Satoh S, Matsumura H, Suzuki F, Hayaishi O (1996) Promotion of sleep mediated by the A2a-adenosine receptor and possible involvement of this receptor in the sleep induced by prostaglandin D2 in rats. Proc Natl Acad Sci U S A 93: 5980-5984.

Sauseng P, Hoppe J, Klimesch W, Gerloff C, Hummel FC (2007) Dissociation of sustained attention from central executive functions: local activity and interregional connectivity in the theta range. Eur J Neurosci 25: 587-593.

Schacter DL (1977) EEG theta waves and psychological phenomena: a review and analysis. Biol Psychol 5: 47-82.

Scharf MT, Naidoo N, Zimmerman JE, Pack AI (2008) The energy hypothesis of sleep revisited. Prog Neurobiol.

Schliebs R, Arendt T (2006) The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. J Neural Transm 113: 1625-1644.

Schurr A (2006) Lactate: the ultimate cerebral oxidative energy substrate? J Cereb Blood Flow Metab 26: 142-152.

Schwierin B, Borbely AA, Tobler I (1996) Effects of N6-cyclopentyladenosine and caffeine on sleep regulation in the rat. Eur J Pharmacol 300: 163-171.

Sebastiao AM, Ribeiro JA (1996) Adenosine A2 receptor-mediated excitatory actions on the nervous system. Prog Neurobiol 48: 167-189.

Segovia G, Porras A, Del Arco A, Mora F (2001) Glutamatergic neurotransmission in aging: a critical perspective. Mech Ageing Dev 122: 1-29.

Seki Y, Feustel PJ, Keller RW, Jr., Tranmer BI, Kimelberg HK (1999) Inhibition of ischemia-induced glutamate release in rat striatum by dihydrokinate and an anion channel blocker. Stroke 30: 433-440.

Semba K (2000) Multiple output pathways of the basal forebrain: organization, chemical heterogeneity, and roles in vigilance. Behav Brain Res 115: 117-141.

Semba K, Reiner PB, McGeer EG, Fibiger HC (1988) Brainstem afferents to the magnocellular basal forebrain studied by axonal transport, immunohistochemistry, and electrophysiology in the rat. J Comp Neurol 267: 433-453.

Semba K, Reiner PB, McGeer EG, Fibiger HC (1989) Brainstem projecting neurons in the rat basal forebrain: neurochemical, topographical, and physiological distinctions from cortically projecting cholinergic neurons. Brain Res Bull 22: 501-509.

Sherin JE, Shiromani PJ, McCarley RW, Saper CB (1996) Activation of ventrolateral preoptic neurons during sleep. Science 271: 216-219.

Shiromani PJ, Lu J, Wagner D, Thakkar J, Greco MA, Basheer R, Thakkar M (2000) Compensatory sleep response to 12 h wakefulness in young and old rats. Am J Physiol Regul Integr Comp Physiol 278: R125-R133.

Shram N, Netchiporouk L, Cespuglio R (2002) Lactate in the brain of the freely moving rat: voltammetric monitoring of the changes related to the sleep-wake states. Eur J Neurosci 16: 461-466.

Sibson NR, Dhankhar A, Mason GF, Rothman DL, Behar KL, Shulman RG (1998) Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. Proc Natl Acad Sci U S A 95: 316-321.

Siles E, Martinez-Lara E, Canuelo A, Sanchez M, Hernandez R, Lopez-Ramos JC, Del Moral ML, Esteban FJ, Blanco S, Pedrosa JA, Rodrigo J, Peinado MA (2002) Age-related changes of the nitric oxide system in the rat brain. Brain Res 956: 385-392.

Sokoloff L (1977) Relation between physiological function and energy metabolism in the central nervous system. J Neurochem 29: 13-26.

Sorg O, Magistretti PJ (1991) Characterization of the glycogenolysis elicited by vasoactive intestinal peptide, noradrenaline and adenosine in primary cultures of mouse cerebral cortical astrocytes. Brain Res 563: 227-233.

Sorg O, Pellerin L, Stolz M, Beggah S, Magistretti PJ (1995) Adenosine triphosphate and arachidonic acid stimulate glycogenolysis in primary cultures of mouse cerebral cortical astrocytes. Neurosci Lett 188: 109-112.

Stenberg D (2007) Neuroanatomy and neurochemistry of sleep. Cell Mol Life Sci 64: 1187-1204.

Stenberg D, Litonius E, Halldner L, Johansson B, Fredholm BB, Porkka-Heiskanen T (2003) Sleep and its homeostatic regulation in mice lacking the adenosine A1 receptor. J Sleep Res 12: 283-290.

Steriade M (2006) Grouping of brain rhythms in corticothalamic systems. Neuroscience 137: 1087-1106.

Steriade M, McCarley R (2005) Brain Control of Wakefulness and Sleep. New York: Kluwer academic/Plenum publishers.

Stone WS (1989) Sleep and aging in animals. Relationships with circadian rhythms and memory. Clin Geriatr Med 5: 363-379.

Strijkstra AM, Beersma DG, Drayer B, Halbesma N, Daan S (2003) Subjective sleepiness correlates negatively with global alpha (8-12 Hz) and positively with central frontal theta (4-8 Hz) frequencies in the human resting awake electroencephalogram. Neurosci Lett 340: 17-20.

Szymusiak R (1995) Magnocellular nuclei of the basal forebrain: substrates of sleep and arousal regulation. Sleep 18: 478-500.

Szymusiak R, Alam N, McGinty D (2000) Discharge patterns of neurons in cholinergic regions of the basal forebrain during waking and sleep. Behav Brain Res 115: 171-182.

Szymusiak R, Alam N, Steininger TL, McGinty D (1998) Sleep-waking discharge patterns of ventrolateral preoptic/anterior hypothalamic neurons in rats. Brain Res 803: 178-188.

Szymusiak R, McGinty D (2008) Hypothalamic regulation of sleep and arousal. Ann N Y Acad Sci 1129: 275-286.

Talk A, Kang E, Gabriel M (2004) Independent generation of theta rhythm in the hippocampus and posterior cingulate cortex. Brain Res 1015: 15-24.

Thakkar MM, Delgiacco RA, Strecker RE, McCarley RW (2003b) Adenosinergic inhibition of basal forebrain wakefulness-active neurons: a simultaneous unit recording and microdialysis study in freely behaving cats. Neuroscience 122: 1107-1113.

Thakkar MM, Engemann SC, Walsh KM, Sahota PK (2008) Adenosine and the homeostatic control of sleep: Effects of A1 receptor blockade in the perifornical lateral hypothalamus on sleep-wakefulness. Neuroscience 153: 875-880.

Thakkar MM, Winston S, McCarley RW (2002) Orexin neurons of the hypothalamus express adenosine A1 receptors. Brain Res 944: 190-194.

Thakkar MM, Winston S, McCarley RW (2003a) A1 receptor and adenosinergic homeostatic regulation of sleep-wakefulness: effects of antisense to the A1 receptor in the cholinergic basal forebrain. J Neurosci 23: 4278-4287.

Ticho SR, Radulovacki M (1991) Role of adenosine in sleep and temperature regulation in the preoptic area of rats. Pharmacol Biochem Behav 40: 33-40.

Timofeev I, Grenier F, Steriade M (2001) Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. Proc Natl Acad Sci U S A 98: 1924-1929.

Tinguely G, Finelli LA, Landolt HP, Borbely AA, Achermann P (2006) Functional EEG topography in sleep and waking: state-dependent and state-independent features. Neuroimage 32: 283-292.

Tobler I, Borbely AA (1990) The effect of 3-h and 6-h sleep deprivation on sleep and EEG spectra of the rat. Behav Brain Res 36: 73-78.

Toescu EC (2005) Normal brain ageing: models and mechanisms. Philos Trans R Soc Lond B Biol Sci 360: 2347-2354.

Toescu EC, Xiong J (2004) Metabolic substrates of neuronal aging. Ann N Y Acad Sci 1019: 19-23.

Tononi G, Cirelli C (2006) Sleep function and synaptic homeostasis. Sleep Med Rev 10: 49-62.

Uehara T, Sumiyoshi T, Itoh H, Kurata K (2008) Lactate production and neurotransmitters; evidence from microdialysis studies. Pharmacol Biochem Behav 90: 273-281.

Urrila AS, Hakkarainen A, Heikkinen S, Vuori K, Stenberg D, Hakkinen AM, Lundbom N, Porkka-Heiskanen T (2003) Metabolic imaging of human cognition: an fMRI/1H-MRS study of brain lactate response to silent word generation. J Cereb Blood Flow Metab 23: 942-948.

Urrila AS, Hakkarainen A, Heikkinen S, Vuori K, Stenberg D, Hakkinen AM, Lundbom N, Porkka-Heiskanen T (2004) Stimulus-induced brain lactate: effects of aging and prolonged wakefulness. J Sleep Res 13: 111-119.

Uylings HB, de Brabander JM (2002) Neuronal changes in normal human aging and Alzheimer's disease. Brain Cogn 49: 268-276.

van der Hiele K., Bollen EL, Vein AA, Reijntjes RH, Westendorp RG, van Buchem MA, Middelkoop HA, van Dijk JG (2008) EEG markers of future cognitive performance in the elderly. J Clin Neurophysiol 25: 83-89.

van Gool WA, Mirmiran M (1983) Age-related changes in the sleep pattern of male adult rats. Brain Res 279: 394-398.

van Lier H, Drinkenburg WH, Coenen AM (2003) Strain differences in hippocampal EEG are related to strain differences in behaviour in rats. Physiol Behav 78: 91-97.

Vanderwolf CH (1969) Hippocampal electrical activity and voluntary movement in the rat. Electroencephalogr Clin Neurophysiol 26: 407-418.

Vanderwolf CH (1975) Neocortical and hippocampal activation relation to behavior: effects of atropine, eserine, phenothiazines, and amphetamine. J Comp Physiol Psychol 88: 300-323.

Vanderwolf CH (1988) Cerebral activity and behavior: control by central cholinergic and serotonergic systems. Int Rev Neurobiol 30: 225-340.

Vanderwolf CH, Raithby A, Snider M, Cristi C, Tanner C (1993) Effects of some cholinergic agonists on neocortical slow wave activity in rats with basal forebrain lesions. Brain Res Bull 31: 515-521.

Vazquez J, Lydic R, Baghdoyan HA (2002) The nitric oxide synthase inhibitor NG-Nitro-L-arginine increases basal forebrain acetylcholine release during sleep and wakefulness. J Neurosci 22: 5597-5605.

Vertes RP (1982) Brain stem generation of the hippocampal EEG. Prog Neurobiol 19: 159-186.

Vertes RP, Hoover WB, Viana DP (2004) Theta rhythm of the hippocampus: subcortical control and functional significance. Behav Cogn Neurosci Rev 3: 173-200.

Vertes RP, Kocsis B (1997) Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. Neuroscience 81: 893-926.

Vincent SR, Williams JA, Reiner PB, el Husseini Ae (1998) Monitoring neuronal NO release in vivo in cerebellum, thalamus and hippocampus. Prog Brain Res 118: 27-35.

Vinogradova OS (1995) Expression, control, and probable functional significance of the neuronal theta-rhythm. Prog Neurobiol 45: 523-583.

Vinogradova OS, Brazhnik ES, Stafekhina VS, Kichigina VF (1995) Modulation of septal influences on hippocampal neurons by cholinergic substances. Neurosci Behav Physiol 25: 453-461.

Vyazovskiy VV, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G (2008) Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nat Neurosci 11: 200-208.

Vyazovskiy VV, Riedner BA, Cirelli C, Tononi G (2007) Sleep homeostasis and cortical synchronization: II. A local field potential study of sleep slow waves in the rat. Sleep 30: 1631-1642.

Vyazovskiy VV, Tobler I (2005) Theta activity in the waking EEG is a marker of sleep propensity in the rat. Brain Res 1050: 64-71.

Winson J (1972) Interspecies differences in the occurrence of theta. Behav Biol 7: 479-487.

Winson J (1978) Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. Science 201: 160-163.

Xie Z, Morgan TE, Rozovsky I, Finch CE (2003) Aging and glial responses to lipopolysaccharide in vitro: greater induction of IL-1 and IL-6, but smaller induction of neurotoxicity. Exp Neurol 182: 135-141.

Young CK, McNaughton N (2008) Coupling of Theta Oscillations between Anterior and Posterior Midline Cortex and with the Hippocampus in Freely Behaving Rats. Cereb Cortex.

Zaborszky L, Gaykema RP, Swanson DJ, Cullinan WE (1997) Cortical input to the basal forebrain. Neuroscience 79: 1051-1078.

Zaborszky L, Pang K, Somogyi J, Nadasdy Z, Kallo I (1999) The basal forebrain corticopetal system revisited. Ann N Y Acad Sci 877: 339-367.

Zimmermann H (2000) Extracellular metabolism of ATP and other nucleotides. Naunyn Schmiedebergs Arch Pharmacol 362: 299-309.